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A POSSIBLE RELATIONSHIP BETWEEN YOLK SIZE AND DIFFERENTIATION IN TROUT EMBRYOS[1]

E. T. GARSIDE AND F. E. J. FRY[2]

Abstract

Myomere numbers were counted in samples of reciprocal hybrids of lake and speckled trout. The fish which developed on speckled trout yolks, which are the smaller, had a lower mean myomere number. Myomere numbers of normal lake trout, speckled trout, and the F_1 hybrid (lake trout egg) were compared with myomere numbers of their respective Siamese twins. An inverse relationship between myomere number and the degree of twinning was found in speckled trout but not in the lake trout or the hybrid. Since this relationship is not evident in fish developed on larger yolks it is assumed that there is a certain critical yolk size below which it becomes limiting and prevents the formation of the normal myomere complement.

Introduction

This study is a preliminary investigation of the possible effect of yolk size, expressed as egg size since there is little difference between them, on differentiation in salmonid embryos. The only reference found thus far concerning the possible influence of yolk on differentiation is a statement by Tåning (2) in which he says that experiments have shown that yolk size does not influence vertebral number. He gives no details of the experiments, so comparison and discussion are not possible.

Materials and Methods

In the first part of this study the myomere numbers were counted for 30 hybrid fry of the cross *Salvelinus fontinalis* (Mitchill), speckled trout, ×*S. namaycush* (Walbaum), lake trout, and 25 fry of the reciprocal cross *namaycush* × *fontinalis*. The two groups were approximately the same age and ranged from 2.5 to 4 cm, standard length. The eggs were incubated at the Laboratory for Experimental Limnology, Maple, Ontario. Incubation and rearing conditions were the same for both groups.

In the second part of the study myomeres were counted in samples of 50 Siamese twins and 50 normal alevins respectively of lake trout, speckled trout,

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Contribution from the Laboratory for Experimental Limnology, which is operated jointly by the Ontario Department of Lands and Forests and the Department of Zoology, University of Toronto, Toronto, Ontario.
[2]Department of Zoology, University of Toronto.

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and their F_1 hybrid, *fontinalis* × *namaycush*, incubated at the Tarentorus Hatchery, Sault Ste. Marie, Ontario. The myomere number of a twinned alevin was computed by adding the mean number of myomeres of the paired portions to the unpaired, posterior number; twins with wholly separate trunks were assigned their mean number. Incubation and rearing conditions were the same for the three groups.

Mean egg diameters were calculated by measuring rows of 10 preserved eggs. Two rows were measured to determine each mean.

Results

Results of myomere counts for the reciprocal cross hybrids are listed in Table I together with mean egg diameters. While the *fontinalis* × *namaycush* hybrid has the larger mean number of myomeres and the larger egg, the difference is not statistically significant.

Table II shows the mean myomere counts for Siamese and normal alevins of the three forms. Significant differences exist among the three groups of normal alevins; the mean counts of the three groups of Siamese alevins differ also significantly from one another. Within each kind the Siamese alevins show significant reductions in their myomere counts as compared with those of the normals, but the reduction is greatest in the speckled trout.

TABLE I

Results of myomere counts and egg measurements in the reciprocal hybrids of the lake trout and speckled trout

Cross	Number in sample	Mean myomere number	Standard error	Egg diameter
S. fontinalis × *C. namaycush*	30	60.8	±0.29	5.2
C. namaycush × *S. fontinalis*	25	59.9	±0.33	4.0

TABLE II

Myomere counts for normal and Siamese twin alevins together with mean egg sizes

Fish	Mean myomere number	Standard error	Egg diameter
Lake trout			
Normal	64.2	±0.44	5.3
Siamese	62.5	±0.35	
Hybrid trout			
Normal	60.1	±0.21	5.3
Siamese	58.8	±0.27	
Speckled trout			
Normal	59.3	±0.20	4.1
Siamese	52.5	±0.44	

Discussion

The possible correlation between myomere number and egg size in the reciprocal hybrids of lake trout and speckled trout prompted additional study to examine further the possible influence of egg size on differentiation. Twinned embryos were selected for this part of the study because of the additional differentiation which takes place with the same amount of yolk as is available to normal embryos. To test this notion the total numbers of myomeres have been plotted against a measure of the degree of twinning, which in this case is the number of unpaired myomeres in the trunks in Fig. 1. The open points are means of data grouped in lots of five. The solid point on each graph is the mean value for the 50 normal alevins. Table III shows the analyses for significance of regression of the lines in Fig. 1. There is a significant regression in the speckled trout data indicating that the total number of myomeres is dependent on the degree of twinning, which is, presumably, a valid, relative measure of the amount of differentiation. Since this relationship is barely evident in the hybrids and nonexistent in lake trout, it is assumed that egg size or more specifically yolk size is in some way responsible for the observed myomere reduction in speckled trout. It is interesting to note that the mean

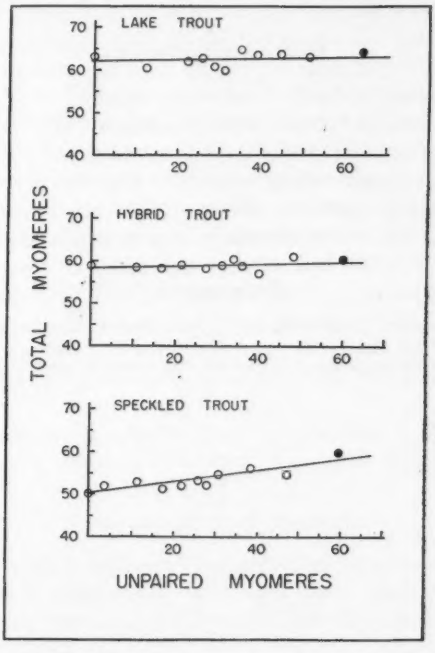

FIG. 1. Relationship of the total number of myomeres to the degree of twinning measured as the number of myomeres in the unpaired trunk. The completely separate twins have the abscissa $x = 0$. Normal alevins are shown by closed circles. Only the speckled trout data have a significant regression.

TABLE III

"t" tests to determine significance of regression of lines in Fig. 1 (from Snedecor (1))

Fish	Slope	Standard error	"t"	D.F.	P
Lake trout	0.037	±0.024	1.54	9	0.1
Hybrid trout	0.040	±0.016	2.50	9	0.05
Speckled trout	0.144	±0.023	5.97	9	<0.01**

myomere number for the hybrid Siamese twins with complete trunks coincides with the mean number for normal speckled trout (59.0 and 59.3 respectively). The mean volume of the lake trout eggs is 73.7 cu. mm and the mean volume of the speckled trout eggs is 38.8 cu. mm. Essentially, the volume of the lake trout yolk has been halved by the two embryos.

The causal mechanism of this phenomenon is not known. It is not probable that such reductions result from any abrupt blockage of differentiating processes since the caudal peduncles and caudal fins have developed normally.

An interesting sequel to this study would be that of the *Prosopium*, *Coregonus*, *Leucichthys* series of eggs which would carry the range of size below that of the speckled trout egg.

Acknowledgments

Eggs of the reciprocal hybrid crosses were supplied by Mr. N. S. Baldwin, Department of Lands and Forests, from Algonquin Park, Ontario. Mr. K. H. Loftus, Department of Lands and Forests, provided the normal and twinned trout alevins from Tarentorus Hatchery, Sault Ste. Marie, Ontario. Financial assistance was received from the National Research Council and from the Ontario Research Foundation, through the University of Toronto Research Committee.

References

1. SNEDECOR, G. W. Statistical methods. 4th ed. Iowa State College Press, Ames, Iowa, U.S.A 1946.
2. TÅNING, A. V. Experimental study of meristic characters in fishes. Biol. Revs. **27**, 169–193 (1951).

A STUDY OF THE TERATOGENIC EFFECTS OF OPENING A WINDOW IN THE SHELL OF THE HEN'S EGG AT 24 HOURS OF INCUBATION[1]

D. J. McCallion and R. B. Clarke

Abstract

A window of about 1 sq. cm in area was cut in the shell of eggs of the domestic hen and the shell membrane removed at about 24 hours of incubation. The square of shell was replaced and sealed with paraffin. The eggs were incubated for a further 24 hours. At that time a large number of the embryos had developed characteristic abnormalities of the central nervous system. These abnormalities result from failure to close of the neural folds and are described by the term acleiencephaly.

Introduction

Ancel (1) has proposed the term acleiencephaly to describe abnormalities in chick embryos in which the neural plate remains open, or is imperfectly closed. Acleiencephaly has been experimentally produced in bird and mammalian embryos by a variety of agents (X rays, Russell (12), Hicks (8); ultra sound, Lutz and Lutz-Ostertag (9, 10); anoxia, Büchner (5), Lutz and Lepy (11); and by several chemical substances, Ancel (1), Giroud et Martinet (7)). In these studies, and in some others (Braverman (3, 4)), when the chick embryo was used, a part of the technique has been the opening of a window in the egg shell and shell membrane with or without the removal of 1–2 cc of albumen.

It is well known that opening a window in the egg shell in order to expose the chorioallantoic membrane for the purpose of making grafts has no apparent effect upon the further development of the host embryo. Similarly, it is possible to make intracoelomic grafts in embryos of about 50 hours of incubation without affecting the development of the host embryos. In the course of a recent investigation (Clarke and McCallion (6)) the authors found that opening a window in the egg shell at about 24 hours of incubation resulted in a very high proportion of embryos with abnormalities of the central nervous system. The occurrence of these abnormalities was so frequent that an alternative method of exposing the young embryos to an agent was used.

In the present paper the results of an investigation of the effects upon the embryo of opening a window in the hen's egg at 24 hours of incubation are reported.

Materials and Methods

Fertile eggs from white Leghorn hens, obtained from a commercial hatchery, were used in this investigation. The eggs were usually incubated within 24 hours of receiving them. Eggs were incubated for 24 hours. At this time

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Contribution from the Department of Zoology, University of Toronto, Toronto, Canada. Supported by a Grant-in-aid of Research from the National Research Council to the first author.

a small hole was drilled in the blunt end of the egg. A 1-cm-square window was cut in the shell with a diamond disk activated by a Foredom dental engine and the egg-shell membrane was removed. The square of shell was replaced and sealed in with paraffin and the small hole at the end of the egg was also sealed with paraffin. In some cases the window was sealed with a square of parafilm. In a number of cases the piece of egg shell was removed and replaced without opening the shell membrane. In others the piece of shell was removed, the shell membrane pricked with a sterile needle, and the egg shell replaced. The eggs were returned to the incubator for a further 24 hours or more. As controls, eggs were incubated for 24 hours, taken from the incubator for a short period of time, and then returned to the incubator for a further 24 hours.

Results of Experiments

The numerical results are tabulated in Table I.

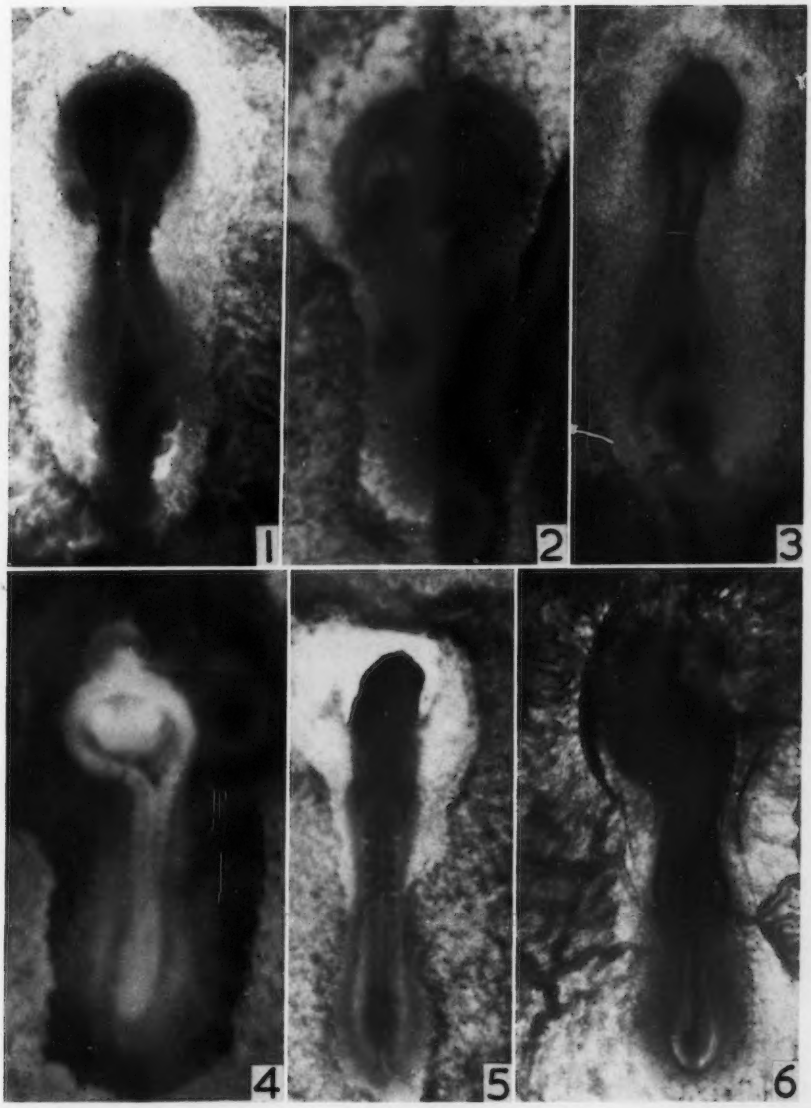
TABLE I

No. of eggs	Treatment	No. of normal embryos	No. of abnormal embryos	% abnormal
256	Controls	240	16	6.2
25	Shell removed and resealed at 24 hours	24	1	4.0
210	Shell and shell membrane removed at 24 hours	85	125	59.2
49	Shell removed and shell membrane pricked at 24 hours	29	20	40.8
143	Shell and shell membrane removed at 48 hours	104	39	27.2

It may be seen from the table that removing the shell alone, without opening the egg-shell membrane, does not affect the further development of the embryo. If, however, the shell membrane is removed, or only pierced, at 24 hours of incubation there is a marked increase in the number of abnormal embryos. The proportion of abnormal embryos is considerably less when the shell membrane is opened at 48 hours of incubation.

The abnormalities produced in these embryos by opening the shell and shell membrane are abnormalities of the central nervous system and are essentially restricted to the anterior end. The abnormalities vary in degree and extent but all are similar in character. The abnormalities can all be classified as some degree of acleiencephaly (Ancel (1)).

In the abnormal embryos the neural folds have incompletely closed or have failed to close anteriorly. The anterior margin of the neural plate is raised up and often pushed posteriorly (Fig. 1). Thus, the heart comes to occupy the most anterior position in the embryo (Figs. 4 and 6). In most cases this results in a bifurcation of the central axis of the embryo (spina bifida) (Figs. 2 and 4). Examples of typical abnormal embryos are shown in Plate I. Various stages in the development of these abnormalities are seen in Figs. 1, 3, 4, and 6.



FIGS. 1–6. Photographs of typical abnormal chick embryos obtained by opening the egg shell and interrupting the egg-shell membrane at 24 hours of incubation. Embryos photographed 24–36 hours after treatment. Note anterior position of heart in Figs. 4 and 6. Fig. 4 unstained; others, borax carmine.

Discussion and Conclusions

The results of these experiments demonstrate quite clearly that opening a window in the shell and shell membrane of the hen's egg at about 24 hours of incubation produces abnormalities of the central nervous system which result from failure to close of the neural plate. The embryos are probably susceptible to this disturbance only within fairly narrow limits at the stages of closure of the neural folds. Since there is a considerable spread in early stages of development at any given hour of incubation this would explain that proportion of embryos that were not affected.

Very little is known of the mechanism of closure of the neural tube except that folding of the neural plate is an active process and also that a certain amount of lateral pressure is exerted by the nonneural ectoderm (Weiss (15)). It is probable that when the egg-shell membrane is removed or pierced the embryo drops somewhat in the egg as a result of changes in the distribution of the egg contents, and that these altered relationships of the embryo thus interfere with the normal mechanism of closure of the neural plate. This does not occur if the shell alone is removed and replaced, but only when the egg-shell membrane is interrupted.

It is rather puzzling that embryos cultured in vitro (Waddington (14), Spratt (13)) develop fairly normally while those remaining in the egg under these conditions develop abnormally. Lutz and Lutz-Ostertag (10) found that exposure of chick embryos in vitro to ultrasonic irradiation produced acleiencephaly while control embryos in vitro were essentially normal. This suggests that the relationships of the embryo are less disturbed on the surface of a culture medium than in the egg but that further disturbance does produce this effect.

In a somewhat similar investigation Ancel (2) removed 2 cc of albumen by means of a syringe through a small hole in the under side of the egg. He then rotated the egg until the hole was above the blastoderm and enlarged the hole to measure 1.5 cm in diameter. He further varied his experiments by placing a drop of distilled water on the exposed blastoderm, or by holding the eggs at room temperature for 48 hours between opening the shell and returning the eggs to the incubator. The lethal effects of these experiments were determined on the basis of the proportion of embryos dying before 72 hours of incubation. The teratogenic effects were determined by the proportion of embryos still alive at 17 days of incubation bearing abnormalities.

It has been demonstrated quite clearly by the present investigation and by that of Ancel that the method of studying the effects of agents injected under the blastoderm after it has been exposed by opening the egg-shell membrane is not acceptable. Our experiments also show that making an injection through the egg-shell membrane is equally unacceptable.

References

1. ANCEL, P. Malformations déterminées par des injections de substances chimiques localisées à la tête d'embryons de poule. J. Embryol. Exptl. Morphol. **3**, 335–357 (1955).
2. ANCEL, P. Recherche sur les effets tératogènes de l'ouverture de l'oeuf de poule au cours des trente-quatre premières heures de l'incubation. Arch. anat. microscop. et morphol. exptl. **45**, 203–217 (1956).

3. BRAVERMAN, M. Neural and mesodermal hierarchies in chick development. Biol. Bull. **115**, 319 (1958*a*).
4. BRAVERMAN, M. An inhibitory extract of chick tissues. Biol. Bull. **115**, 344 (1958*b*).
5. BÜCHNER, F. Differenzierungsstörungen im mittleren und hinteren Kopfdrittel des Hühnchens nach experimentellem Sauerstoffmangel. Beitr. pathol. Anat. **115**, 617–643 (1955).
6. CLARKE, R. B. and MCCALLION, D. J. Specific inhibition of neural differentiation in the chick embryo. Can. J. Zool. **37**, 133–136 (1959).
7. GIROUD, A. et MARTINET, M. Morphogenèse de l'anencéphalie. Arch. anat. microbiol. **46**, 247–264 (1957).
8. HICKS, S. P. Developmental malformation produced by radiation. Am. J. Roentgenol. **69**, 272–293 (1953).
9. LUTZ, H. et LUTZ-OSTERTAG, Y. Action des ultra-sons sur le développement du blastoderme non incubé d'oiseau. Arch. anat. microbiol. **46**, 307–324 (1957).
10. LUTZ, H. et LUTZ-OSTERTAG, Y. Acléiencéphalie expérimentale *in vitro*. J. Embryol. Exptl. Morphol. **6**, 504–509 (1958).
11. LUTZ, H. et LEPY, M. Action du gaz carbonique sur la blastoderme non incubé d'oiseau Bull. soc. zool. France, **83**, 76–81 (1958).
12. RUSSELL, L. B. X-ray induced developmental abnormalities in the mouse and their use in the analysis of embryological patterns. J. Exptl. Zool. **114**, 545–602 (1950).
13. SPRATT, N. T. Normal development in extravital culture of chick blastoderm. J. Exptl. Zool. **106**, 345 (1947).
14. WADDINGTON, C. H. Experiments on the development of chick and duck embryos cultivated *in vitro*. Phil. Trans. Ser. B, **211**, 179–230 (1932).
15. WEISS, P. Principles of development. Henry Holt and Co., New York. 1939.

WATER ABSORPTION BY THE EGGS OF CRICKETS[1]

J. E. McFarlane, A. S. K. Ghouri,[2] and C. P. Kennard[3]

Abstract

Absorption of water by the eggs of *Acheta configuratus*, *Gryllodes sigillatus*, and a Canadian and a Pakistani strain of *Acheta domesticus* takes place during early embryonic development. The amount of water absorbed varies from about 60 to 120% of the weight of newly laid eggs, depending on the species or strain. The time of absorption is generally earlier the more rapid is embryonic development, indicating that the embryonic stage during which water is absorbed is similar in all forms. Absorption is more rapid at 33 than at 28° C. In all forms, a small but rapid loss in water occurs just after maximal absorption; this loss takes place also when the eggs are immersed in distilled water. The incubation periods of the four forms at various temperatures (28–38° C) are presented.

Introduction

The physiology of development of the eggs of crickets is largely unknown. The only cricket species which has been studied critically in this regard is *Acheta commodus* (Walker), studied by Browning (3, 4, 5, 6), although Busvine (7) has presented some observations on *Acheta domesticus* (L.). The present article deals with the water relations and effect of temperature on the rate of development of the eggs of the following four 'forms' of crickets (Orthoptera : Gryllidae): a Canadian and a Pakistani strain of *Acheta domesticus* (L.), which have been previously shown to be reproductively isolated from each other (9); *A. configuratus* (Walker), a field species native to the Indian subcontinent (8); and *Gryllodes sigillatus* (Walker).

Methods

The source and maintenance of cultures of the insects have been described in an earlier paper (10). Stock adults were maintained at 28° C, and nymphs at 35° C.

Eggs were obtained by placing small dishes, filled with moist sand, into the jars containing the adult cultures. These egg dishes, after removal from the jars, were emptied on paper towelling and air-dried at room temperature for about half an hour until the eggs had separated from the sand particles. The eggs were then picked up with a fine moist brush, completely freed of sand particles by sieving under water, and then examined for defective eggs under a binocular microscope. The eggs used in any single experiment were obtained from cultures having from 10 to 30 females.

The eggs were incubated in air-tight jars on a piece of moist filter paper resting on a slightly moist (not wet) pad of absorbent cotton. The jars were

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Contribution from the Department of Entomology, Macdonald College, McGill University, Macdonald College P.O., Que.
[2]Department of Plant Protection, Government of Pakistan, Karachi.
[3]Central Agricultural Station, Mon Repos, British Guiana.

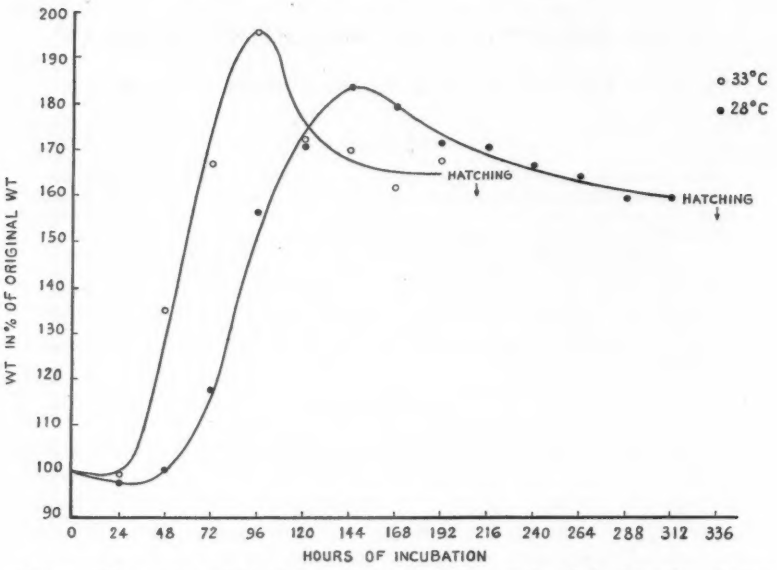


FIG. 1. Change in weight of eggs of the Canadian strain of *A. domesticus* during incubation at 28° and 33° C. Each point represents the weight of 100 eggs.

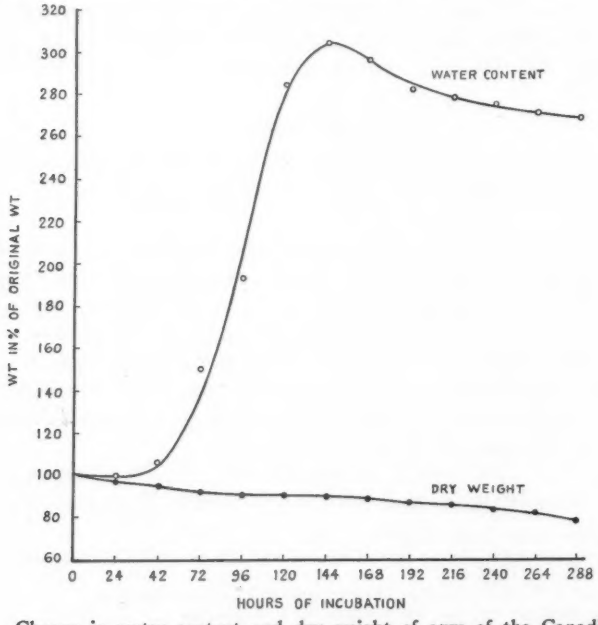


FIG. 2. Change in water content and dry weight of eggs of the Canadian strain of *A. domesticus* during incubation at 28°C. Each point represents the weight of 100 eggs.

placed in incubators at 28°, 33°, 35°, or 38° C, or in water baths at 28°, 33°, or 38° C. The maximal temperature variation in the water baths was ±0.5° C, in the incubators ±1° C.

For the study of the effect of temperature on rate of development the water baths were used, except for the test at 35° C. For each experiment, eggs of one or more forms, and of the Canadian strain of *A. domesticus*, which served as a control, were obtained from egg dishes which had been placed in the adult cultures 12 hours previously; therefore at the time of removal of the dishes no egg was more than 12 hours old. The eggs were cleaned and counted during the next 12 hours, being kept at room temperature during this time, and then incubated* in three water baths at different constant temperatures. The eggs in each jar were examined at least 12 hours before the expected time of first hatching at a given temperature. The emergence of hoppers was recorded every 12 hours thereafter until hatching was over, the hoppers being removed at each observation.

To determine the change in weight during the course of development, the eggs of the four forms were obtained within 12 hours after they were laid, and, after thorough washing, followed by removal of surface moisture with filter paper, were weighed, in lots of 50 or 100, on a direct-reading balance.† Each lot was then transferred to moist filter paper and cotton in an air-tight jar, and incubated in a water bath, incubation being started 12 hours after removal of the egg dishes from the cultures. At prearranged intervals, one jar, at random, was removed from the water bath and the eggs weighed as before.

Observations

1. *Water Absorption*

The data are expressed as weight in per cent of original weight (i.e. of newly laid eggs), no absolute weights being given. The newly laid egg of the Canadian strain of *A. domesticus* weighs about 0.33 mg, that of the Pakistani strain 0.25 mg, of *G. sigillatus* 0.41 mg, and of *A. configuratus* 0.32 mg.

(*a*) *A. domesticus, Canadian strain.*—The change in wet weight of the eggs during incubation on moist filter paper at 33° and at 28° C is presented in Fig. 1. At 33° C, the weight began to increase at about 36 hours, reached a maximum of about 196% after 96 hours, declined rapidly to about 175% after 120 hours, and then more slowly to about 165% at the time of hatching. At 28° C, weight increase began at about 48 hours; the weight reached a maximum of about 184% after 144 hours, declined rapidly for the next 48 hours, and thereafter more slowly to about 161% at the time of hatching. Figure 2 shows the change in water content and dry weight at 28° C (the change in wet weight of the same eggs is plotted in Fig. 1) expressed as percentage of the original water content and dry weight. The dry weight decreased at a uniform rate during incubation to about 80% at the time of hatching. The

*This step marks the beginning of incubation and is the 'zero' time referred to in this paper. The term 'incubation period' as used in this work is the time required for the completion of embryonic development *after* the eggs had been placed at the experimental temperatures, and not the total time elapsed between laying and hatching.

†Spoerhase Model 10M, Cave & Co., Vancouver, B.C.

changes in water content, however, paralleled the changes in wet weight (Fig. 1). The water content of the newly laid eggs was 43.1%. Figure 3 shows the change in wet weight of the eggs immersed in distilled water during incubation at 33° C. At about 36 hours the eggs began to increase in weight, reaching a maximum of 170% at 120 hours (12 hours later than the eggs incubated on moist filter paper), then declined in weight to about 155% at the time of hatching.

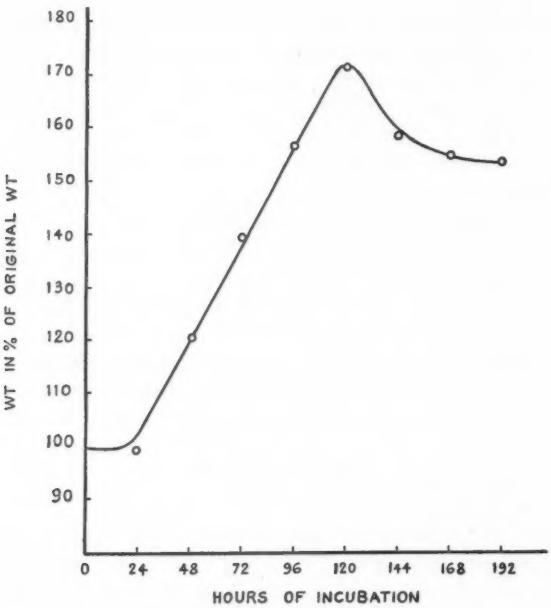


FIG. 3. Change in weight of eggs of the Canadian strain of *A. domesticus* immersed in distilled water during incubation at 33° C. Each point represents the weight of 50 eggs.

(*b*) *A. domesticus, Pakistani strain.*—The change in wet weight of the eggs during incubation in moist filter paper at 33° C is shown in Fig. 4. The weight began to increase at about 24 hours, reached a maximum of 218% after 48 hours, then declined rapidly to about 190% after 84 hours, remaining at that level until hatching.

(*c*) *A. configuratus.*—Figure 5 shows the change in wet weight during incubation on moist filter paper at 33° C. At about 36 hours, the weight began to increase, the maximum weight of 190% was reached at about 130 hours, and thereafter the weight declined to about 170% at hatching.

(*d*) *G. sigillatus.*—The change in wet weight of eggs incubated on moist filter paper at 33° C is shown in Fig. 4. The weight began to increase at about 48 hours, reaching a maximum of 162% at 72 hours, and thereafter declining to 140% at the time of hatching.

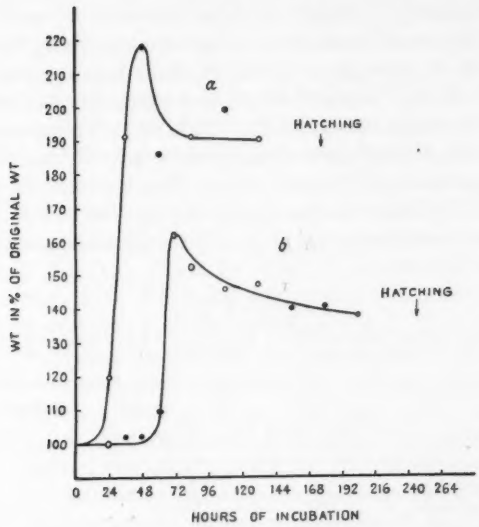


FIG. 4. Change in weight during incubation on moist filter paper of eggs of (*a*) the Pakistani strain of *A. domesticus*, and (*b*) *G. sigillatus*. Open circles = 100 eggs, closed circles = 50 eggs.

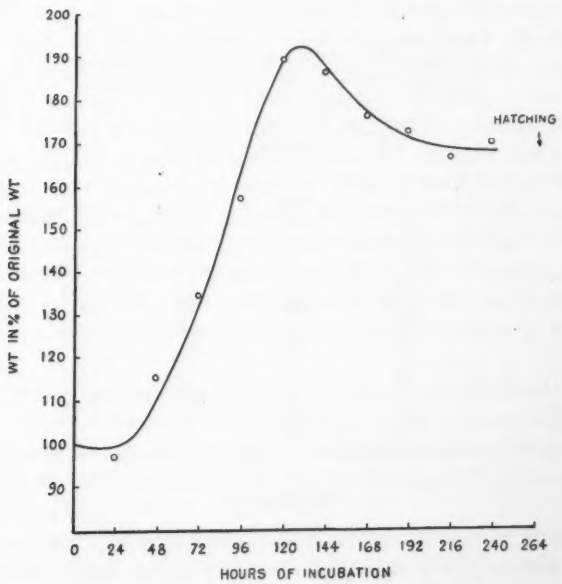


FIG. 5. Change in weight of eggs of *A. configuratus* during incubation at 33° C. Each point represents the weight of 100 eggs.

2. *Incubation Periods*

Table I gives the mean incubation period and percentage hatch of eggs of the two strains of *A. domesticus* incubated simultaneously at 28°, 33°, and 38° C. The eggs of the Pakistani strain had a significantly greater speed of development at the three temperatures ($P<0.01$). There was no significant difference in the rate of development within a strain at 33° and 38° C ($P>0.05$).

The percentage hatch and mean incubation period of the eggs of *A. configuratus* and *G. sigillatus* at four temperatures (28°–38° C), as compared with those of the Canadian strain of *A. domesticus* simultaneously incubated, are presented in Table II.

TABLE I

Incubation period and percentage hatch of eggs of the Canadian and Pakistani strains of *A. domesticus* at three temperatures

Temp. (° C)	Strain	No. eggs incubated	% hatch	Incubation period (days)	
				Mean	Range
28	Canadian	200	76	13.6	12.5–16.0
	Pakistani	200	70	10.8	10.0–12.0
33	Canadian	150	72	9.1	8.5–10.5
	Pakistani	297	64	7.3	6.5–11.0
38	Canadian	100	76	9.2	8.5–10.0
	Pakistani	250	74	7.1	6.5– 8.5

TABLE II

Incubation period and percentage hatch of eggs of *A. domesticus* (Canadian), *G. sigillatus*, and *A. configuratus* at four temperatures

Species	Temp. (° C)	No eggs incubated	% hatch	Incubation period (days)	
				Mean	Range
A. domesticus (Canadian)	28	190	80	13.6	12.5–16.0
	33	315	74	9.4	8.5–11.5
	35	150	66	8.8	8.5–10.5
	38	200	58	9.0	8.5– 9.5
G. sigillatus	28	250	72	14.9	13.5–18.5
	33	250	72	10.0	9.0–12.0
	35	100	78	9.0	8.5–10.0
	38	200	64	9.0	8.5–11.0
A. configuratus	28	250	74	15.7	14.5–20.0
	33	500	72	11.8	10.5–13.5
	35	150	80	11.2	10.0–13.0
	38	300	0	—	—

Discussion

Browning's are the only observations on water absorption by cricket eggs which have hitherto been reported (6). His curves describing the rate of water absorption in *Acheta commodus* at various temperatures are, not unexpectedly, sigmoid, as are those for the four forms studied in the present

work, and also those for a number of other insects (1, 6, 12). Browning also found that the rate of absorption varied with the temperature, the rate being slowest at 20.9°, more rapid at 26.5°, and most rapid at 29.9° C. Our results (Fig. 1) with the Canadian strain of *A. domesticus*, showing that the rate of absorption was more rapid at 33° than at 28° C, thus conform with Browning's.

The eggs of all four forms in the present study declined in weight rather abruptly during incubation at 33° C just after the maximal weight had been reached (Figs. 1, 4, 5); the eggs of the Canadian strain of *A. domesticus* incubated at 28° C also showed a decline in weight at this stage, but the decline was more gradual (Fig. 1). That this drop in weight is due mainly to a decrease in water content of the eggs is evident from Fig. 2 (which shows also that the increase in weight is due to water absorption). The decline in weight occurs also when the eggs are immersed in distilled water (Fig. 2).

Browning (6) found that newly laid eggs of *Acheta commodus*, directly incubated, lost weight at 29.9° and 26.5° C, just after maximal weight had been reached; eggs incubated at 20.9° C did not show this decline, but rather a steady increase and then a levelling off, the final weight attained being about the same as that of the eggs incubated at 29.9° C; and the eggs incubated at 26.5° C showed a second minor increase in weight that occurred just after the loss in weight. On the other hand, newly laid eggs which had been transferred to 13° C for varying periods of time and then incubated at the above three temperatures showed a very slight, scarcely detectable, loss in weight after maximal absorption had occurred, and the disparity between the rate of absorption at 20.9° and at the higher temperatures was diminished.

Although the eggs of the Canadian strain of *A. domesticus* absorbed water more rapidly at 33° than at 28° C, and *apparently* absorbed a greater amount of water, the water loss was more rapid at the higher temperature, and the amount of water lost was *apparently* greater, so that the final weight, or weight just before hatching, was about the same at both temperatures. This is what one might expect, assuming that the same amount of water is taken up and lost by the eggs at both temperatures: there is more rapid development and less variation in rate of development at the higher temperature (Table I), so that the curve for water absorption might be expected to be further to the left, to rise more steeply and decline more rapidly, as compared with the 28° curve.

According to this assumption, there should be a lower temperature at which the curve for water absorption should show a steady increase with no water loss being perceptible. In Browning's curves for water absorption by directly incubated eggs of *Acheta commodus*, there is no decline at 20.9° such as occurs at 26.5° and 29.9° C.

It should be noted that the *maximal* drop in weight (from peak weight to the last measurement) found by Browning was about 6%, at 29.9° C (estimated from his graph). This is about half (11–14%) of the drop we have found. However, one must consider the fact that all the eggs of the forms studied in

this work did not hatch; indeed the average hatch is about 70% for all forms (Tables I and II). Of the eggs which do not hatch, some do not develop at all, others develop apparently normally, but fail to hatch, while the majority die during the course of development. As the eggs that die lose part of their liquid contents (the filter paper becomes stained) and therefore principally water (at least when incubated on filter paper), the effect of the death of eggs on the average weight would be greater, and perhaps only detectable, when the eggs died after the period of water absorption. However, although the death of eggs may contribute to the total water loss, it is unlikely that it can account for the timing or for the rapid rate of loss.

In this connection, it may be a significant fact that the newly laid weight of eggs of *Acheta commodus* is about double that of the forms studied in the present work, which means that the absolute weights of water lost are much closer than the percentages. In any case, the fact that water is lost at all, however slight the loss may be, is significant in that it represents loss against a concentration gradient. A possible explanation for this loss in water is discussed elsewhere (11).

From a comparison of Figs. 1 and 3 it is apparent that water is taken up less rapidly when the eggs are immersed in distilled water than when they are placed on moist filter paper. This is probably due to a relative deficiency in oxygen of the eggs in the water. Beament (2) has reported the unpublished observation of Browning that eggs of *Acheta commodus* are able to develop and hatch when immersed in distilled water.

The fact that water is taken up at about the same relative time in development, with the maximum weight being reached earliest in the eggs of the Pakistani strain of *A. domesticus*, which develop most rapidly, and latest in the eggs of *A. configuratus*, which develop least rapidly, suggests that water is absorbed at the same stage of development in all four forms (Tables I and II). This stage has been identified in the Canadian strain of *A. domesticus* as that during which the egg is enclosed in the serosa (11).

The newly laid eggs of *G. sigillatus* are perhaps more fully hydrated than those of the other forms, as they are heavier and take up less water. Conversely, the eggs of the Pakistani strain may be less fully hydrated.

A comparison of the incubation periods of the Canadian and Pakistani strains of *A. domesticus* (Table I) further illustrates the distinctness of the two forms (9,10).

References

1. BANKS, C. J. The absorption of water by the eggs of *Corixa punctata* Illig (Hemiptera–Corixidae) under experimental conditions. J. Exptl. Biol. **26**, 131–136 (1949).
2. BEAMENT, J. W. L. Water transport in insects. Symposium Soc. Exptl. Biol. **8**, 94–117 (1954).
3. BROWNING, T. O. The influence of temperature on the rate of development of insects, with special reference to the eggs of *Gryllulus commodus* Walker. Australian J. Sci. Research, B, **5**, 96–111 (1952).
4. BROWNING, T. O. The influence of temperature on the completion of diapause in the eggs of *Gryllulus commodus* Walker. Australian J. Sci. Research, B, **5**, 112–127 (1952).

5. BROWNING, T. O. On the rate of completion of diapause development at constant temperatures in the eggs of *Gryllulus commodus* Walk. Australian J. Sci. Research, B, **5**, 344–353 (1952).
6. BROWNING, T. O. The influence of temperature and moisture on the uptake and loss of water in the eggs of *Gryllulus commodus* Walker (Orthoptera–Gryllidae). J. Exptl. Biol. **30**, 104–115 (1953).
7. BUSVINE, J. R. Simple methods for rearing the cricket (*Gryllulus domesticus* L.) with some observations on speed of development at different temperatures. Proc. Roy. Entomol. Soc. London, A, **30**, 15–18 (1955).
8. CHOPARD, L. *In* La biologie des Orthoptères. *Edited by* Paul Lechevalier et fils, Paris. 1938.
9. GHOURI, A. S. K. and McFARLANE, J. E. Reproductive isolation in the house cricket. Psyche, **64**, 30–36 (1958).
10. GHOURI, A. S. K. and McFARLANE, J. E. Observations on the development of crickets. Can. Entomologist, **90**, 158–165 (1958).
11. McFARLANE, J. E. and KENNARD, C. P. Further observations on water absorption by the eggs of *Acheta domesticus* (L.). (In preparation.)
12. SALT, R. W. Water uptake in the eggs of *Melanoplus bivittatus* (Say). Can. J. Research, D, **27**, 236–246 (1949).

MORE SHELL FIGURES AND NOTES ON BARNACLES[1]

IRA E. CORNWALL[2]

Abstract

More of the figures in the wall plates of sessile barnacles are shown and an English name, interlaminate figures, is suggested for them. It has been found that there are patterns, or markings, in the ala of the wall plates that could be used to identify some of the species that do not have figures in the wall plates. Several of these patterns are shown and the method of finding them is described.

Material

The material for this paper has been received from the following: Dr. C. H. Edmondson, Director of the Bernice P. Bishop Museum, Honolulu, Hawaii; Dr. Dora P. Henry of the Department of Oceanography, University of Washington, Seattle, Wash.; The U.S. National Museum, Washington, D.C.; Dr. L. B. Holthuis of the Rijkmuseum van Natuurijke Historie, Holland; Dr. D. J. Crisp, Director of the Marine Biology Station, Menai Bridge, Anglesay, North Wales; The Allan Hancock Foundation, Los Angeles, California; R. B. Lyons of the University of Oregon Medical School, Portland, Oregon; The British Museum (Natural History), London, England; Dr. L. G. Hartlein of the California Academy of Sciences, Golden Gate Park, San Francisco, California; and personal collecting.

Discussion

There now appears to be no doubt that the figures in the shell of sessile barnacles are true indicators of species, and there also is no doubt that by using the figures some former identifications will prove to be incorrect. In getting together the material for this and my earlier paper (4) several cases of this nature were brought to light. For example, some specimens sent to me were labeled *Balanus concavus pacificus*, but the figure in their shells are not like the figures of that species; one is shown in Fig. 1 and is the same as the figure in *B. decorus* (Fig. 2). Evidently they should be identified as *B. decorus*.

Since 1895, when Alessandri (1) found the figures in the shell, very little work has been done on them. Gruvel (7) shows a few in his monograph and Madame Davadie-Suaudeau (6) shows a few more in her work on the Algerian fossil barnacles. Both these works are in French and the French term for the figures is "lames hypodermiques." There does not seem to be any English equivalent for this, so the name interlaminate figures is suggested herewith.

[1]Manuscript received January 15, 1959.
[2]Address: 1951 Argyle Avenue, Victoria, B.C.

The shell of the sessile barnacle is unique as it is the only shell, as far as is known, that has in itself a mark, or design, by which the species can be identified. There are a few species of sessile barnacles in which no figures have been found, but most that have been examined have them. Some of the figures are large, well marked, and easy to see; others are faint and hard to see. For example, the figures in *B. decorus* (Figs. 1–2) were difficult to see. Even the application of benzyl alcohol, as recommended by Dr. J. P. Harding of the British Museum, failed to make the figures visible. But it was found that turning the specimen so that the light struck it at a different angle caused the figures to appear. The best angle can only be found by turning the stage of the microscope till the figures can be seen.

Another factor that adds greatly to the difficulty of identifying barnacles is the great ability of the shell to take different forms when the specimens are crowded. *Balanus cariosus* (Figs. 6–9) is a good example of this. The normal shape of an uncrowded specimen is a steep-walled cone (Fig. 6) covered with downward-pointing spines, giving it what Darwin called a "thatched appearance". This kind of specimen is rare. On the British Columbia coast this species covers the rocks a little above low-tide mark. Those growing on rocks that are exposed to much wave action are forced to take another form. There the shells are thick, grow close together, and the walls are coalesced. Apparently the rubbing of the driftwood wears the shell down, but the cover plates yield to a certain extent and are not eroded and consequently project above the shell (Fig. 7). When they grow on the piling of a wharf the shells form long thin tubes that are packed close together, each may be 2 or 3 inches long (Fig. 8). If the barnacle grows on the point of a rock it can grow only in one direction. Here it forms a thick-walled tube and rarely develops any spines (Fig. 9). Judging from their outside appearance these four specimens appear to be four different species, but an examination of the inside sculpture of the cover plates, the mouth parts, and the cirri shows that they are all the same species.

The writer found a fossil barnacle in the Sooke Formation in 1927, and described it as a new species (5). It was named *Balanus connelli* (Fig. 10) in honor of the late Rev. Robert Connell who had done so much work on the fossils of the Sooke Formation. But recent research has shown that this barnacle is not a new species, but is without doubt *B. tintinnabulum* (Fig. 11). Furthermore the outline of the undistorted pores in both specimens is roughly oval and has a peculiar indentation just above where the figure commences. This is clearly another case in which a knowledge of the interlaminate figures would have aided in making a correct identification.

The encroachment of the parietal pores by the figure has been noticed in several species. For instance, in the fossil *B. perforatus* (Fig. 17) the figure forms nearly the whole side of the parietal pore. In *B. regalis* (Fig. 12) it is just the opposite as the outline of the pore seems to limit the branches of the figure.

Balanus galeatus (Fig. 15) is a small barnacle usually found on the stem of a gorgonian. Although several sections were made of the one examined,

only a series of white dots could be seen. In some parts of the shell the dots were arranged in pairs and in other parts they were in double rows. This little barnacle is easily recognized as it looks like a miniature boat with part of the keel divided and grasping the stem of the gorgonian.

The markings in the shell of *Balanus rostratus* (Figs. 3–5) are quite distinctive, but can hardly be called figures as they are just lines between the pores. Some of the lines are straight and some curved. There are several subspecies of *rostratus* and many have been examined to try and find some variation in the figure that would distinguish the subspecies, but without success.

Balanus regalis (Figs. 12–13) is a large barnacle with large parietal pores that have cross-septa from base to apex. The external surface of the wall is finely ribbed and the ribs are large near the base. The exterior of the wall plates is "pink to Vandyke red clouded with white" (8). The interior of the shell is pink. The interlaminate figure (Figs. 12–13) is very distinctive. The stem of the figure extends to the outer lamina and in the specimen examined there were 14, or more, branches. Between some of the branches there is a fine white line and many of the branches have a similar line running through the center. The part of the shell in which the figure lies is lighter than the rest of the shell.

A section of a wall plate of *Verruca stromia* (Fig. 14) has a peculiar appearance. It is permeated by minute pores that do not reach either the outer or inner lamina. These pores are irregularly arranged and are of various lengths. The genus *Verruca* Schumacher has been found in some ancient formations. It is a peculiar-looking shell as two of the cover plates are stationary and form part of the wall. According to Withers (9, p. 323) it appears to be a matter of chance whether the right-hand or the left-hand scuta and terga are modified to form the wall with the rostrum and carina.

While examining a specimen of *Balanus amphitrite* from Vineyard Sound, Mass., a peculiar pattern was found (Fig. 18). It is not in the wall plate like the interlaminate figure but is in the ala. The ala is the part of the edge of the wall plate that projects *under* the adjacent plate (see Fig. 25, B and B). The finding of this pattern suggests it might be useful in identification of some of those species that do not have interlaminate figures. Here is a case where that could be done: *Balanus cariosus* does not have an interlaminate figure, but it has a figure in the ala (Fig. 22) which is quite characteristic of the species. Another species in which an interlaminate figure has not been found is *B. engbergi*. It also has a figure in the ala (Fig. 24). It seems a good idea to show as many as possible of the patterns found in the ala as a fragment of an ala may be found that requires identification.

The interlaminate figures are found by cutting a wall plate at right angles to the line of growth as shown by line A–A in Fig. 25. The line of growth of the wall plates is vertical, that is from the base to the apex of the shell. In an undistorted specimen the cut is made nearly parallel to the base, and this is quite a simple matter. But to find the ala pattern is a little more

difficult. The line of growth of this small part of the wall plate is at right angles to the line of growth of the main plate, therefore the cut must be made vertically as shown at B and B in Fig. 25. The best way to prepare the shell to see the ala patterns is to boil it in potassium hydroxide and take it apart. Then the ala of each plate can be examined. If the shell is small and delicate each plate should be embedded in a block of plaster of Paris, as described in my previous papers (3 and 4, p. 80).

So far none of the ala patterns are symmetrical like the interlaminate figures but are very irregular. Yet in spite of their great irregularity there are certain features about each one that are definitely characteristic of the species. For instance the pattern found in the ala of *Balanus amphitrite* (Fig. 18) consists of a row of figures that have several protuberances on *one* side only. The ala pattern of *B. nubilus* (Fig. 23) has figures no two of which are alike. *B. crenatus* has a row of upright irregular figures, some are narrow, some broad, and they are not all placed at the same angle. *B. altissimus* (Fig. 19) has the most irregular pattern of any examined. The patterns in the ala of *B. concavus pacificus* consist of a row of irregular light dots. Forty were counted in one specimen examined, shown in Fig. 20. *B. cariosus* (Fig. 22), like other barnacles with membranous base, has no interlaminate figure, but it has a well-marked pattern in the ala. *B. engbergi* has no interlaminate figure and the ala pattern (Fig. 24) is quite faint. All the alae of several specimens were examined but only one had a pattern in it. Two sections were made of this ala and the pattern could be seen in both.

Unfortunately there does not seem to be anything about a barnacle that indicates its age. If it has a leaf-like figure such as *Balanus concavus* (Fig. 5) the figure increases in size as the barnacle grows. If the figure has branches such as *B. decorus* (Figs. 1–2) then the number of branches increases as the shell grows. This can be seen by making a section at the top of the shell which will show the figure as it was when the shell was small. A series of sections made at different places in the wall will show the gradual increase in the size of the figure, or the increase in the number of branches, as the shell grows. It seems possible that the number of branches of the figure may indicate the number of growth periods, but nothing certain is known about this.

Dr. E. L. Bousfield (2) gives the age of several Atlantic species. *Balanus improvisus* lives only 2 years, *B. hameri* 8 years, *B. balanus* 7, *B. balanoides* 6, and *B. crenatus* 4 years.

There does not seem to be any record of the ages of Pacific barnacles. For several years I collected *B. nubilus* from one place. This species is found only below low-tide mark. It grows to a very large size, some specimens being 100 mm in diameter and about 75 mm high. They grow in clusters and sometimes on a large specimen there is a whole series of young ones ranging from small ones just beginning to form a shell to large ones with eggs in them. If each of these represents a spawning season then *B. nubilus* must live for at least 5 years or more.

References

1. ALESSANDRI, G. Contribuzione allo studio dei cirripedi fossili d'Italia. Boll. Soc. Geol. Italiana Roma, **13**, 234–314 (1895).
2. BOUSFIELD, E. The distribution and spawning seasons of barnacles on the Atlantic Coast of Canada. Natl. Museum Can. Bull. 132 (1953).
3. CORNWALL, I. E. Identifying fossil and recent barnacles by the figures in the shell. J. Paleontol. **30**, 646–651 (1956).
4. CORNWALL, I. E. Identifying recent and fossil barnacles. Can. J. Zool. **36**, 79–89 (1958).
5. CORNWALL, I. E. Fossil Cirripedia from the Upper Oligocene Sooke formation of Vancouver Island, B.C. Univ. Calif. Publs. Geol. Sci. **16**, 399–408 (1927).
6. DAVADIE-SUAUDEAU, C. Contribution à l'étude des balanides tertiaires de l'Algérie. Bull. Serv. Carte Géol. Algérie, Ser. 1, Paléontol. No. 14, 36–43 (1952).
7. GRUVEL, A. Monographie des cirrhipèdes ou thécostraces. Libraires Acad. Méd. Paris, 365–371 (1905).
8. PILSBRY, H. A. The sessile barnacles (Cirripedia) contained in the collections of the U.S. National Museum, including a monograph. U.S. Natl. Museum Bull. 93 (1916).
9. WITHERS, T. H. Catalogue of fossil cirripedia. British Museum (Nat. Hist.). Vol. 2. 1928.

EXPLANATION OF FIGURES

PLATE I

FIG. 1. *Balanus decorus* Darwin. From Huntington Beach, California. Collected at Velero Station No. 1126-40 of the Allan Hancock Foundation. This was wrongly identified as *B. concavus pacificus*. Identified by I. E. Cornwall.

FIG. 2. *B. decorus*. A small pink barnacle sent to me by Dr. C. H. Edmondson, Director of the Bernice P. Bishop Museum, Honolulu. Identified by Dr. Dora P. Henry.

FIG. 3. *B. rostratus* Hoek. U.S. National Museum specimen No. 85961 from Labrador, collected by R. A. Bartlett. Identified by I. E. Cornwall.

FIG. 4. *B. rostratus*. Fossil, collected from the Sooke Oligocene Formation, Vancouver Island, B.C. Identified by I. E. Cornwall.

FIG. 5. *B. concavus* Bronn. Fossil. U.S. National Museum specimen, from Miocene Formation, Scientists Cliff, Chesapeake Bay, Md. Identified by I. E. Cornwall.

FIG. 6. *B. cariosus* (Pallas). Normal specimen, cone-shaped, covered with downward-pointing spines. From William Head, Vancouver Island, B.C. Identified by I. E. Cornwall.

FIG. 7. *B. cariosus*. One from a large group with driftwood-worn shells. Shells are thick and coalesced. From William Head, B.C. Identified by I. E. Cornwall.

FIG. 8. *B. cariosus*. Thin-walled specimens growing close together on the piles of a wharf. From William Head, B.C. Identified by I. E. Cornwall.

FIG. 9. *B. cariosus*. A specimen growing on the point of a rock. Thick walls and no trace of spines. From William Head, B.C. Identified by I. E. Cornwall.

FIG. 10. *B. connelli* Cornwall. Fossil. From the Oligocene Sooke Formation, Vancouver Island, B.C. The finding of the interlaminate figures showed that this is *B. tintinnabulum* and not a new species. Identified by I. E. Cornwall.

FIG. 11. *B. tintinnabulum californicus* Pilsbry. From Pacific Grove, California. For comparison with No. 10. Identified by I. E. Cornwall.

PLATE II

FIG. 12. *B. regalis* Pilsbry. From Santa Maria Bay, Lower California; Mexico. Collected from Velero Station No. 127-33 of the Hancock Foundation. Identified by Dr. G. P. Ashcraft.

FIG. 13. Another part of shell No. 12.

FIG. 14. *Verruca stromia* (O. F. Müller). U.S. National Museum specimen No. 12076. No locality given with this specimen. Identified by I. E. Cornwall.

FIG. 15. *Balanus galeatus* (Linnaeus). U.S. National Museum specimen No. 48046, from South Carolina, growing on gorgonian. Identified by H. A. Pilsbry.

FIG. 16. *B. perforatus* Bruguière. From Bay of Naples. Identified by Dr. L. B. Holthuis.

FIG. 17. *B. perforatus*. Fossil. British Museum specimen. From Middle Pliocene, Walton-on-Naze, England. Identified by I. E. Cornwall.

FIG. 18. Pattern found in ala of *B. amphitrite* from Vineyard Sound, Mass. Identified by I. E. Cornwall.

PLATE III

FIG. 19. *B. altissimus* Cornwall. From William Head, Vancouver Island, B.C. Section through ala. Figures plainly marked. Identified by I. E. Cornwall.

FIG. 20. *B. concavus pacificus* Pilsbry. Fossil. U.S. National Museum specimen No. 13111, from Crescent City. Section through ala. Identified by H. A. Pilsbry.

FIG. 21. *B. crenatus* Bruguière. From ship in Esquimalt Harbor. Identified by I. E. Cornwall.

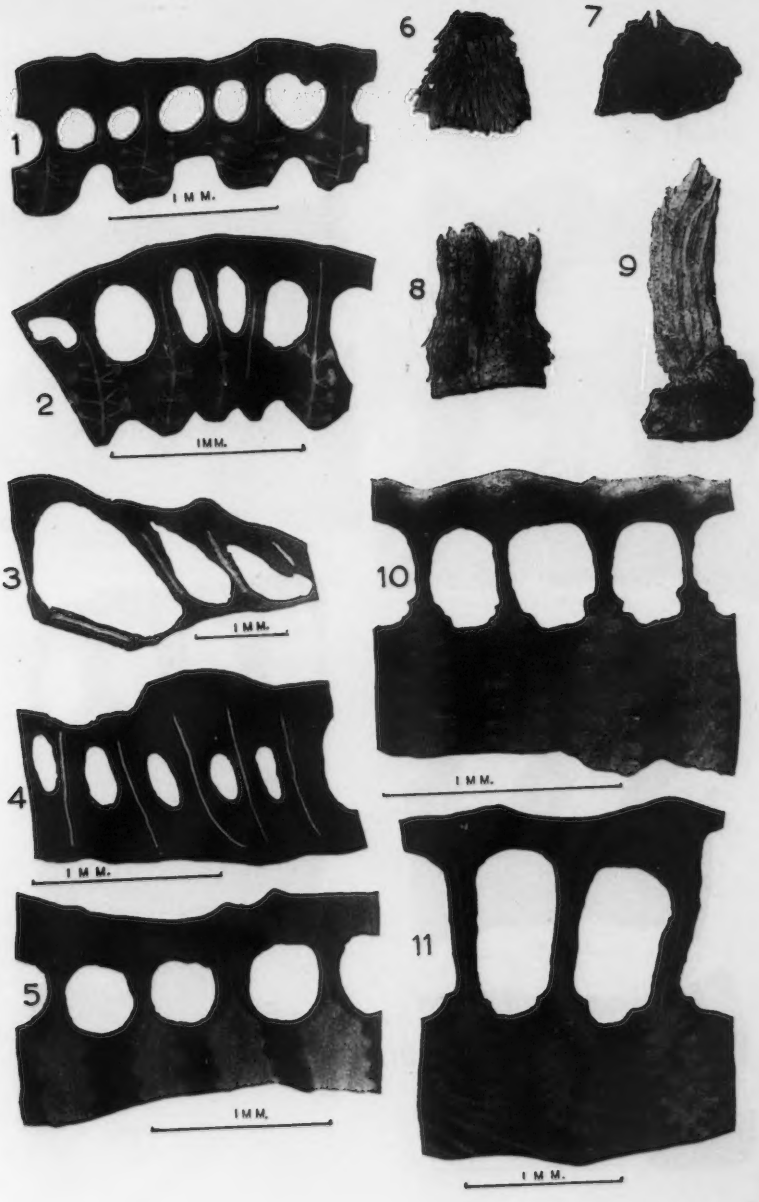
FIG. 22. *B. cariosus* (Pallas). From ship in Esquimalt Harbor. Section through ala. Identified by I. E. Cornwall.

FIG. 23. *B. nubilus* Darwin. From Oregon Coast, collected by R. B. Lyons. Identified by I. E. Cornwall. Section through ala. Figure very faint.

FIG. 24. *B. enbergi* Pilsbry. San Juan Island, Washington. Identified by Dr. Dora P. Henry. Section through ala.

FIG. 25. Barnacle shell. Cut is made at A–A to see interlaminate figures. Cut made at B and B to see pattern in ala.

NOTE: Figs. 1–25 follow.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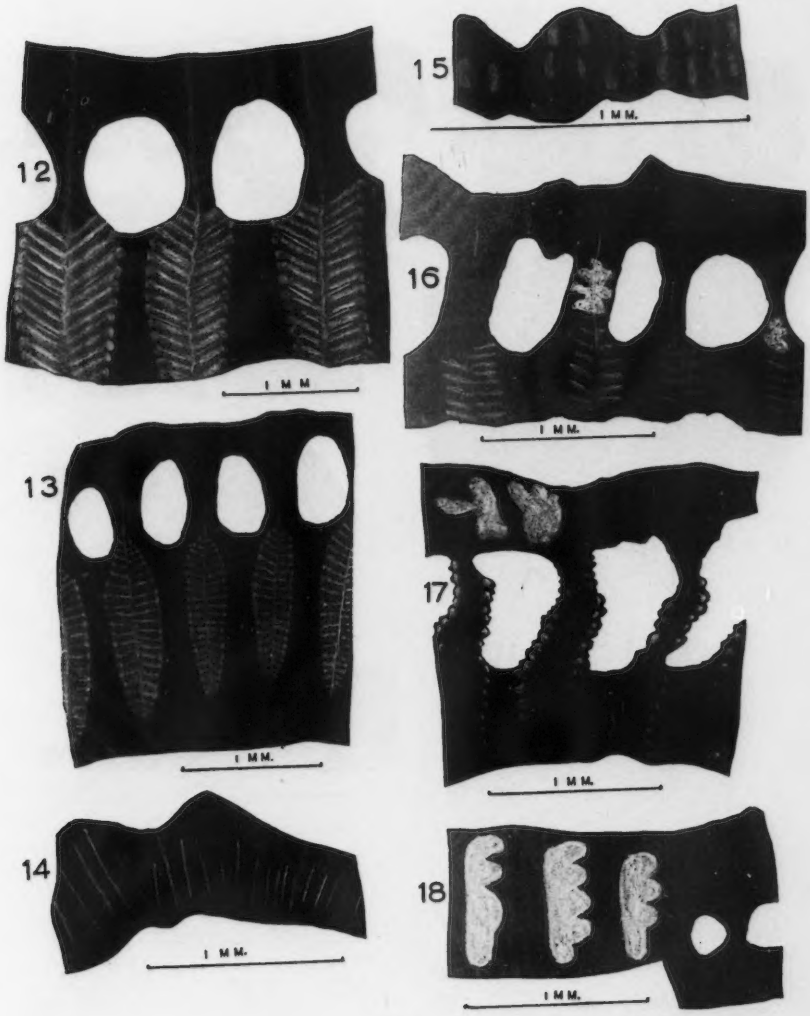
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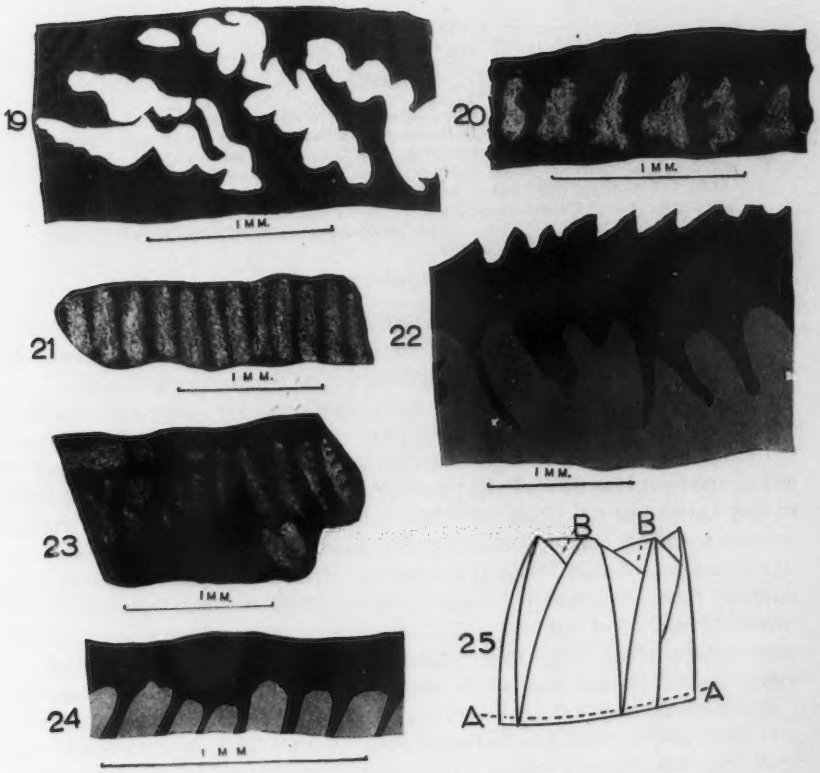
PLATE III



THE EGG AND FIRST-STAGE LARVA OF DESMIDOCERCELLA NUMIDICA (SEURAT, 1920) WITH REMARKS ON THE AFFINITIES OF THE DESMIDOCERCIDAE[1]

ROY C. ANDERSON

Abstract

Mature females of *Desmidocercella numidica* (Seurat, 1920) have been found in a great blue heron (*Ardea herodius*) captured in Algonquin Park on May 30, 1955. As far as can be determined, this represents the first discovery of mature females of this species. The egg and first-stage larva are described and the affinities of the Desmidocercidae are considered in the light of these new data. In particular, the numerous resemblances of the desmidocercids to the Aproctinae and the Tetracheilonematinae are pointed out.

Introduction

The classification of the Filarioidea has, in recent years, undergone considerable modification with the increase in knowledge of the life cycles and morphology of the group. The morphology of the first-stage larva often has an important bearing on the classification. Thus, those filarioids with microfilarioid first-stage larvae (incompletely differentiated and accumulating in the tissues of the definitive host) are embraced by the Onchocercidae and the Setariidae and those in which the larvae are fully formed and pass out of the host are placed in the Diplotriaenidae and Filariidae. An important gap in our knowledge of the larvae of filarioids has been provided by the species of the exceedingly curious and little-understood family Desmidocercidae Cram, 1927. The major difficulty has been that mature females of the most common species *Desmidocercella numidica* (Seurat, 1920) have not been found. Seurat (15) declared as early as 1920 that "l'existence de cette filaire adulte, mais toujours rencontrée à l'état immature dans les sacs aériens du Héron cendré soulève, au point de vue biologique, un problème des plus intéressants". Since this time numerous authors have reported and often redescribed this species, generally under different names,* and in nearly every instance they reported the females as immature as judged from the absence of eggs or larvae. An exception appears to be that of Singh (16), who reported small eggs without larvae in specimens from *Ardeola grayi* in India. In contrast the closely related species *D. incognita* (Skrjabin) of *Phalocrocorax carbo* was described by Ssolonitzin (21) as having eggs with thick, smooth shells, 44 × 26 μ in size, and Dubinina and Serkova (9) stated that specimens from

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Contribution from the Department of Parasitology, Ontario Research Foundation, Toronto 5. Prof. Alain A. Chabaud, Institut de Parasitologie, Paris, kindly reviewed the manuscript.

*The synonymies proposed by Chabaud, 1957 (2) are followed throughout. Chabaud recognized only three species of *Desmidocercella*, viz. *D. numidica*, *D. incognita* Ssolonitzin, 1932, and *D. australis* Mawson, 1953. In addition, the genus *Desmidocerca* contains the single species *D. aerophila* Skrjabin, 1916 and *Diomedenema* the single species *D. diomedeae* Johnston and Mawson, 1952. For a list of references to *D. numidica* the reader is referred to Chabaud's paper.

Phalacrocorax pygmaes were mature. Similarly the specimens of *Diomedenema diomedeae* were said by Johnston and Mawson (12) to contain eggs 23×58 μ in size and Skrjabin (17) reported eggs 30×18 μ in size in *Desmidocerca aerophila* from *Ardea cineria* and *Phalacrocorax carbo*.

During the summer of 1955 five adult *Ardea herodius* from Algonquin Park, Ontario, were autopsied. Two were captured on May 17, one on May 30, one on July 17, and one on July 26. Specimens of *D. numidica* were found in the abdominal and thoracic air sacs of one of the herons collected on May 17 (nematode collection, Ontario Research Foundation No. 25) and the one collected on May 30 (nematode collection Nos. 24, 26). The nematodes collected on May 17 did not contain eggs with larvae and were presumed immature. The adult female heron autopsied on May 30, on the other hand, contained five female worms, the uteri and vaginae of which were packed with larvated eggs.* The latter broke easily when pressed gently under a cover slip, and the larvae, after stretching themselves, remained immobile in physiological saline. Preliminary examination of the fresh larvae revealed that they had an oesophagus, intestine, and rectum (viz. "differentiated"). No spines such as are found in the larvae of diplotriaenids were observed. The larvae and adults were subsequently preserved in glycerine–alcohol.

The author has only recently had an opportunity to re-examine these preserved specimens and, although further study of living material will undoubtedly shed more light on the morphology of the larvae of the Desmidocercidae, it is felt that these observations are worth publishing inasmuch as mature females of *D. numidica* are evidently found so rarely and the morphology of the egg and larva seems to shed some light on the affinities of the group.

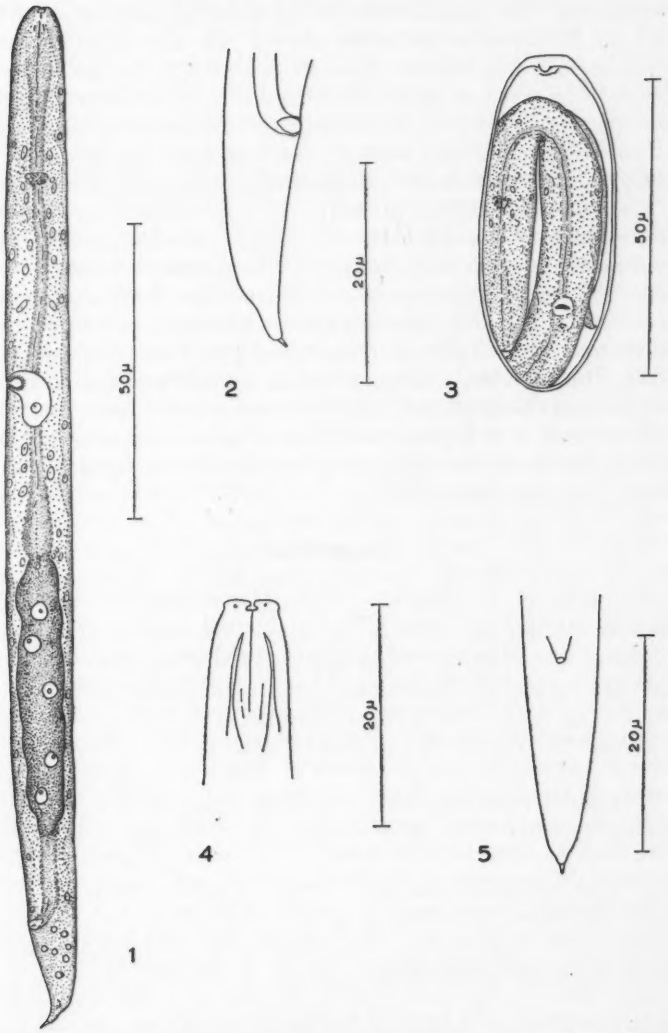
Methods

The eggs removed from the vagina and upper parts of the uterus of one female were studied in water, pure glycerine, or lactophenol with cotton blue. The latter mixture was especially useful as the lactophenol softened the shell of the eggs, which were then easily broken by gentle pressing and rolling under the cover slip. In this way many nicely stained, intact larvae were obtained for prolonged study. Many dozens of such larvae were studied and sketched after which a generalized illustration was made (Fig. 1).

Description

The elongate eggs (Fig. 3) are smooth and thin-shelled, and 56 by 24 μ in size. The shell at one end of the egg is slightly flat and thicker than elsewhere, its inner surface bearing a rounded, refractory protuberance. The larvae (Figs. 1–2, 4–5) is 170–176 μ in length and 9–10 μ in maximum width.

*The five mature females measured 5.2–6.0 mm in length and 0.28–0.31 mm in width as compared to 5.0–5.8 mm by 0.24–0.27 mm for the immature specimens. The other dimensions were also extremely similar. Other dimensions of the mature specimens were as follows: length muscular oesophagus 0.18–0.22 mm; length glandular oesophagus 0.41–0.50 mm; vulva 0.56–0.63 mm from cephalic extremity; nerve ring 0.12–0.13 mm from cephalic extremity; length tail 0.069–0.078 mm.



FIGS. 1–5. *Desmidocercella numidica* (Seurat, 1920). FIG. 1. Diagram first-stage larva from egg, lateral view. FIG. 2. Caudal extremity first-stage larva, lateral view. FIG. 3. Egg from *vagina vera* of female. FIG. 4. Anterior extremity first-stage larva, lateral view. FIG. 5. Caudal extremity first-stage larva, ventral view.

Its anterior extremity is somewhat flat and in a few larvae is slightly constricted 1 or 2 μ behind the stoma. The stoma is exceedingly minute and enters a small but well-defined cavity. A pair of dark spots are present at the extremity which may represent amphids or papillae. The oesophagus, 89–97 μ in length, is well developed but the details of its composition could not be

fully ascertained. The anterior part of the oesophagus seems to be slightly expanded and the posterior end is club-shaped. The nerve ring is exceedingly difficult to see but was present about 27 μ from the anterior extremity in three larvae. The excretory pore, a well-defined laterally running slit 59–65 μ from the anterior extremity, leads into a prominent, globular vesicle surrounded by a radially striated band of cytoplasm from the excretory cell, the nucleus and main body of which lies generally behind, and to the side of, the pore. The intestine, 40–50 μ in length, is most distinct and seems to be composed of five or six large cells as judged from the presence of large, unstained nuclei in a columnar mass of deeply staining cytoplasmic material (note that the cotton blue usually stained only the cytoplasm of the cells, leaving the nuclei as clear spherical bodies containing refractory nucleoli). The rectum and the anal pore are inconspicuous and were observed in only a few larvae. In these the anal pore was on a slight swelling and the inner cuticular lining of the distal end of the rectum was fairly clear. The tail of the larva, about 23–29 μ in length, terminates in an undulation and a spike-like structure (cf. the first-stage larvae of protostrongylid nematodes). A genital primordium was not observed.

Discussion

The life cycles of the members of the Desmidocercidae have not been satisfactorily elucidated. Dubinin (8) found larvae in the eyes of small fishes in the Volga delta and he claimed that these developed into adult *D. numidica* when fed to the appropriate species of birds. If this finding is well-founded, one may expect to find that some aquatic arthropod eaten by the fish acts as intermediate host after ingesting the larvae evacuated by the definitive host.

A further unusual feature of reports of desmidocercids is found in their localization in the definitive host. In Ontario *D. numidica* has been found only in the air sacs of *Ardea herodius* but others have recorded it in the heart (18), gall bladder (9), and kidneys and lungs (14). Similarly Gushanskaya (10) reported *D. incognita* in the trachea, heart, gall bladder, stomach, and intestines although it was originally reported by Ssolonitzin (21) in the air sacs. The air sacs of birds are much more complex than is generally believed and conceivably specimens thought to be in the pericardium, or on the intestines or kidneys, were, in fact, in the air sacs; naturally, it is not surprising to find such worms in the lungs or trachea, if the air sacs are the usual site. The possibility that these nematodes wander after the death of the host should also not be overlooked. Nevertheless, all such reports should not be discounted for some filarioids may reach the air sacs via the circulatory system, and there seems little reason for not believing that they could live in organs associated with the alimentary tract.

The position of the family Desmidocercidae within the Spirurina has posed a perplexing problem for many years. Yorke and Maplestone (23) placed the genera *Desmidocerca* and *Desmidocercella* in the Thelaziidae of the Spiruroidea. Baylis and Daubney (1) placed *Desmidocerca* in the Spiruridae. Cram (7),

in proposing the family Desmidocercidae, included it in the Spiruroidea as did Chitwood and Wehr (6). Wehr (22), Chitwood and Chitwood (5), Chabaud and Choquet (4), and most recently Chabaud and Anderson (3) placed it among the Filarioidea. Soviet helminthologists (e.g. 11, 19, 20) have generally preferred to include it in the Spiruroidea. López-Neyra (13) excluded the Desmidocercidae from his monograph on the Filarioidea. Dubinin (8) transferred the genus *Desmidocercella* to the filarioids while leaving the genus *Desmidocerca* in the spiruroids.

The absence in the Desmidocercidae of pseudolabia or structures that might be considered homologous with them, together with the absence of the internal circle of cephalic papillae (except for the internolaterals which Chitwood and Wehr, 1934 have reported in *D. numidica;* these papillae also persist in certain species of filarioids), indicate affinities with the Filarioidea. In addition, the localization of these nematodes, generally in the air sacs of birds, suggests a possible relationship between them and the Diplotriaenidae or the Filariidae. However, the absence of highly developed circumoral or trident-like structures shows that they are not closely related to either the Diplotriaeninae or the Dicheilonematinae. This conclusion is well borne out by the structure of the first-stage larva of diplotriaenids the tail of which is blunt, rounded, and surrounded by a circlet of minute spines; several rows of spines are found as well at the anterior end. These features are entirely different from those found in the larva of *D. numidica.*

Turning to the Filariidae, one finds no close relationship between the desmidocercids and the Filariinae, the latter, characterized notably by a parastomal vulva, being subcutaneous parasites of mammals. On the other hand, there are a number of striking, and possibly significant, similarities between the Desmidocercidae, the Aproctinae, and the Tetracheilonematinae. They are as follows:

1. They are parasites predominantly of the respiratory passages of birds; some species of *Aprocta* are known from the air sacs and nasal passages as well as from the orbit.

2. Their size is comparable to that of some members of the Aproctinae.

3. The form of the caudal extremity in both sexes (short, rounded, without terminolateral papillae) with its exceedingly conspicuous dorsorectal muscle (*depressor ani*) is very similar to that found in the Aproctinae and the Tetracheilonematinae.

4. The highly variable oesophagus, excessively long in *Desmidocerca aerophila* but shorter and sometimes divided in species of *Desmidocercella*, is comparable to that found in the Aproctinae (cf. *Aprocta papillosa* and *A. fiorii*).

5. The cylindrical, thick-walled buccal cavity is reminiscent of that found in *Mawsonfilaria;* the buccal capsule of *Squamofilaria* is also highly developed.

6. The size, shape, and structure of the egg capsule bears a striking resemblance to that found in the Aproctinae and the Tetracheilonematinae.

7. The morphology of the larva is similar to that of the Aproctinae and the Tetracheilonematinae. One notes especially the pointed, unarmed tail and

the absence of spines at the anterior end. Unfortunately the larva of only *Aprocta fiorii* Chabaud and Anderson, 1959 has been described and its tail is much attenuated. However, the tail of the larva of *Tetracheilonema quadrilabratum* (Molin, 1858) is, according to López-Neyra (13), similar to that of *D. numidica*.

There are apparently four major differences between the Desmidocercidae, and the Aproctinae and Tetracheilonematinae.

1. The spicules are short and equal in size and morphology in the Aproctinae and the Tetracheilonematinae but markedly dissimilar in size and form in the Desmidocercidae.

2. A median, preanal papilla, found in the males of the Aproctinae and Tetracheilonematinae, has not been reported in the Desmidocercidae.

3. The pair of lateral, trilobed, tooth-like structures present in the stoma of *D. numidica* and *Diomedenema diomedeae*, although undescribed in other members of the family, is unknown in the Aproctinae or the Tetracheilonematinae.

4. The presence of spines or tubercle-like structures on the caudal extremities of *D. aerophila*, *D. australis*, and *D. incognita*, but absent in *D. numidica*, are unknown in the Aproctinae and the Tetracheilonematinae (they occur in *Filaria* of the Filariinae, however).

The position of the vulva is highly variable in the Desmidocercidae. Thus, although in the posterior half of the body in *D. aerophila*, it is anterior to the junction of the oesophagus and intestine in *D. numidica*. In *D. incognita* it is median in position. The position of the vulva, therefore, neither adds nor detracts from one's views regarding the affinities of the Desmidocercidae with the Aproctinae group. The vulva is equatorial or posterior to the middle of the body in the Oswaldofilariinae of the Filarioidea showing that a posterior position of the vulva does not preclude the possibility of including a nematode in the Filarioidea.

In summary, the similarities between the desmidocercids and the Aproctinae group seem to far outweigh their differences and, in fact, there might be some justification for placing the two in the same family in the future especially as the Aproctinae are clearly unrelated to the Filariinae with which they are presently associated in the Filariidae. More information on the ecology of the desmidocercids would be desirable however.

References

1. BAYLIS, H. A. and DAUBNEY, R. A synopsis of the families and genera of Nematoda. British Museum, London. 1926.
2. CHABAUD, A. G. Note sur les nématodes du genre *Desmidocercella*. Ann. parasitol. humaine et comparée, **32**, 343–347 (1957).
3. CHABAUD, A. G. and ANDERSON, R. C. Nouvel essai de classification des filaires (superfamille des Filarioidea). II. Ann. parasitol. humaine et comparée, **34**, 64–87 (1959).
4. CHABAUD, A. G. and CHOQUET, M. T. Nouvel essai de classification des filaires (superfamille de Filarioidea). Ann. parasitol. humaine et comparée, **28**, 172–192 (1953).
5. CHITWOOD, B. G. and CHITWOOD, M. B. An introduction to nematology. Sect. 1. Anatomy. Monumental Printing Co., Baltimore. 1950.

6. CHITWOOD, B. G. and WEHR, E. E. The value of cephalic structures as characters in nematode classification, with special reference to the superfamily Spiruroidea. Z. Parasitenk. **7**, 273–335 (1934).
7. CRAM, E. B. Bird parasites of the nematode suborders Strongylata, Ascaridata and Spirurata. Bull. No. 140. U.S. Natl. Museum. 1927.
8. DUBININ, V. B. [Experimental studies on the life cycles of some parasitic worms in animals of the Volga delta] (in Russian). Parasitologich. Sbornik, **11**, 145–151 (1949).
9. DUBININA, M. N. and SERKOVA, O. P. [Nematodes of the birds wintering in southern Tadzhikistan] (in Russian). Parasitologich. Sbornik, **13**, 75–95 (1951).
10. GUSHANSKAYA, L. C. [Studies on the Spirurata of water birds in the U.S.S.R.] (in Russian). Trudi Gelminthol. Lab. Akad. Nauk, SSSR, **4**, 55–63 (1950).
11. Guschanskaya, L. C. [The family Desmidocercidae Cram, 1927 and its place in the systematics of the Nematoda.] (in Russian.) Helm. works presented to K. I. Skrjabin on his 75th birthday anniversary. Moscow. 1953. pp. 188–210.
12. JOHNSTON, T. M. and MAWSON, P. M. Some nematodes from Australian birds and mammals. Trans. Roy. Soc. S. Australia, **75**, 30–37 (1952).
13. LÓPEZ-NEYRA, C. R. Revision de la superfamilia Filarioidea (Weinland, 1858). Rev. ibérica parasitol. **16**, 3–225 (1956).
14. OSHMARIN, P. A. and BELOUS, E. V. [Notes on filariae of wild animals] (in Russian). Trudi Gelminthol. Lab. Akad. Nauk, S.S.S.R. **5**, 121–127 (1951).
15. SEURAT, L. G. Sur une filaire du héron cendré. Bull. soc. hist. nat. Afrique Nord, **11**, 142 (1920).
16. SINGH, S. N. Studies on the helminth parasites of birds in Hyderabad State. Nematoda, I. J. Helminthol. **22**, 77–92 (1948).
17. SKRJABIN, K. I. Nématodes des oiseaux du Turkestan russe. Ann. Mus. Zool. Acad. Imp. Sci. Petrograd, **20**, 457–557 (1916).
18. SKRJABIN, K. I. [Beitrag zur Kenntnis der Vogelfilarian Russlands] (in Russian). Rev. Microbiol. Saratov, **2**, 90–91 (1923).
19. SKRJABIN, K. I. and SHIKHOBALOVA, N. P. [Filariae of animals and man] (in Russian). Moscow. 1948.
20. SKRJABIN, K. I., SHIKHOBALOVA, N. P., and SOBOLEV, A. A. [The determination of parasitic nematodes] (in Russian). Moscow. 1949.
21. SSOLONITZIN, I. A. Ueber zwei neue Arten von Nematoden im Vogel. Zentr. Bakteriol. Parasitenk. Abt. Orig. **124**, 361–365 (1932).
22. WEHR, E. E. A revised classification of the nematode superfamily Filarioidea. Proc. Helminthol. Soc. Wash. D.C. **6**, 95–97 (1935).
23. YORKE, W. and MAPLESTONE, P. A. The nematode parasites of vertebrates. Whitefriars Press Ltd., London. 1926.

A CANADIAN STRAIN OF ENTAMOEBA MOSHKOVSKII CHALAIA, 1941[1]

Brother PHILIPPE J. LACHANCE

Abstract

A strain of *Entamoeba moshkovskii*, designated the F.I.C. strain, has been isolated from sewage material at Ste. Anne de Bellevue, Province of Quebec, Canada.

Casagrandi and Barbagallo (3), considering the parasitic amoebae generically distinct from the free-living ones, established the genus *Entamoeba* for the large nonpathogenic amoeba of the human intestine, which Grassi (7) had described as *Amoeba coli*. Some 50 species of *Entamoeba* are now recognized, among them *E. histolytica*, the dysentery amoeba of man; *E. gingivalis*, the mouth amoeba of man; and *E. invadens*, a pathogenic amoeba of some reptiles.

The genus was long thought to comprise only endoparasitic (but not necessarily pathogenic) species. Recent editions of some well-known textbooks still hold this to be so. However, the existence of a free-living *Entamoeba* was revealed 18 years ago, when Chalaia (4) described *E. moshkovskii* from a Moscow sewage-disposal plant.

This amoeba is morphologically indistinguishable from *E. histolytica*. As far as its biology is known, it is an anaerobic free-living polysaprobe. All attempts to infect man and laboratory animals with it have failed. The places and habitats from which *E. moshkovskii* has been recorded are listed in Table I.

TABLE I

Known distribution and habitats of *E. moshkovskii*

Author(s)	Date	Place	Ecological niche
Chalaia (4)	1941	Moscow, U.S.S.R.	Sewage
Wright *et al.* (10)	1942	California, U.S.A.	Sewage
Gnezdilov (6)	1947	Leningrad, U.S.S.R.	Sewage
Chalaia (5)	1947	Moscow, U.S.S.R.	Sewage, ponds
		Minsk, U.S.S.R.	River
Amaral and Azzi Leal (1)	1949	São Paulo, Brazil	Sewage
Neal (8)	1950	London, England	Sewage
Rogava (9)	1958	Tashkent, U.S.S.R.	Irrigation channels, ponds

As part of current studies of *Entamoeba*, it was decided to search for this organism in sewage effluent from Ste. Anne de Bellevue, Province of Quebec, Canada. The first sample, when subcultured, yielded a strain of *E. moshkovskii*.

[1]Manuscript received April 24, 1959.
Contribution from the Institute of Parasitology, McGill University, Macdonald College P.O., Que., Canada, with financial assistance from the National Research Council of Canada.

The sewage-disposal plant in question receives material from the town of Ste. Annes, from Macdonald College of McGill University, and from Ste. Annes Military Hospital.

Each Friday the material in the septic tank is pumped into the Ottawa River, running nearby, and the sides and bottom of the tank are then washed with hose and water.

On August 15, 1958, after the tank had been emptied but before it had been washed out, material was scraped from its sides. This semiliquid material had a strong soapy smell, was slightly alkaline (pH 7.5), and its temperature was 20° C.

The sediment obtained by centrifuging the material contained an abundant bacterial flora, various flagellates and ciliates, rotifers, nematode larvae, and fungi. It was used as inoculum for various culture media, and at different temperatures.

Best results were obtained from cultures grown at 23° C, in Balamuth's medium (2). Besides 5 ml of the egg yolk infusion, each culture tube contained some sterile starch and three drops of an aqueous solution of penicillin G and dihydrostreptomycin (concentration, 10,000 I.U. and 10 mg respectively, per ml). After 2 weeks these cultures contained a small number of amoebae and a few clumps of cysts. On staining with Lugol's iodine, some of the cysts were found to be quadrinucleate. In subsequent subcultures the numbers of trophozoites and cysts increased, and today, 9 months after having been collected, the organism continues to multiply and encyst at 23° C.

At 35° C, and even at 37° C, this strain multiplies, but less abundantly than at 23° C, and does not encyst. When cultured at high temperatures it requires frequent transfers.

The following methods were employed in an attempt to purify the strain:

(1) addition of the penicillin–streptomycin solution mentioned above, on two occasions,

(2) a 1-hour exposure at 37° C,

(3) a 16-minute treatment with aqueous phenol (0.1% w/v).

Of all the other protozoa present in the original sample of sewage material only flagellates remained numerous in the subcultures.

During the course of this work a sample of organisms, drawn from each of five successive subcultures, was stained with Lugol's iodine, and 100 cysts measured from it. The resultant arithmetic means (Table II) are smaller than some of the averages reported by Neal (8) but nearly coincide with the figure (10 μ) given by Chalaia (4).

These physiological and ecological data, as well as the morphological characters of both fresh and stained material, are in full agreement with Chalaia's description (4) of *E. moshkovskii*.

In homage to the Founders and Members of the Teaching Order to which I belong, I propose to designate this strain of *E. moshkovskii* the F.I.C. strain. F.I.C. is the official abbreviation of the Latin name (Fratres ab Instructione Christiana) for the Order of the Brothers of Christian Instruction.

TABLE II

Cyst measurements from successive subcultures (F.I.C. strain)

Class mid-point, μ	Successive subcultures					
	1st	2nd	3rd	4th	5th	Total
7.4	1	1	2	2	1	7
8.7	5	4	16	20	18	63
9.9	32	44	59	63	51	249
11.1	57	49	21	14	29	170
12.4	4	2	2	1	1	10
13.6	1	0	0	0	0	1
Mean ± standard error (μ)	10.64±.09	10.47±.08	9.96±.09	9.80±.08	10.03±.09	10.18±.09
Standard deviation (μ)	±0.89	±0.80	±0.88	±0.82	±0.89	±0.91

Acknowledgments

I wish to express my gratitude to Mr. W. Lavigne, Town Engineer of Ste. Annes, and to Mr. A. Perreault for their collaboration, as well as to Drs. R. A. Neal and E. Meerovitch for helpful suggestions.

References

1. Amaral, A. D. F. and Azzi Leal, R. Sobre uma *Entamoeba* semelhante à *E. histolytica* encontrada em material de esgôto. Rev. paulista med. **34**, 173–176 (1949).
2. Balamuth, W. Improved egg-yolk infusion for cultivation of *Entamoeba histolytica* and other intestinal protozoa. Am. J. Clin. Pathol. **16**, 380–384 (1946).
3. Casagrandi, O. and Barbagallo, P. Ricerche biologiche e cliniche sull'*Amoeba coli* (Lösch). Seconda e ultima nota preliminare. Boll. accad. Gioenia Sci. Nat. Catania, **41**, 7–19 (1895).
4. Chalaia, L. E. (On a species of *Entamoeba* detected in sewage.) (In Russian.) Med. Parazitol. Parazitar. Bolezni, **10**, 244–252 (1941).
5. Chalaia, L. E. (Contribution to the study of *Entamoeba moshkovskii*.) (In Russian.) Med. Parazitol. Parazitar. Bolezni, **16**, 66–69 (1947).
6. Gnezdilov, V. G. (The geographical distribution, epidemiology and prophylaxis of amoebiasis.) (Russian text.) Med. Parazitol. Parazitar. Bolezni, **16**, 13–32 (1947).
7. Grassi, G. B. Dei protozoi parassiti e specialmente di quelli che sono nell'uomo. Gazz. med. Italo-Lombard, **39**, 445–448 (1879).
8. Neal, R. A. A species of *Entamoeba* from sewage. Trans. Roy. Soc. Trop. Med. Hyg. **44**, 9 (1950).
9. Rogava, L. I. (On *Entamoeba moshkovskii*.) (Russian text, English summary.) Med. Parazitol. Parazitar. Bolezni, **27**, 330–334 (1958).
10. Wright, W. H., Cram, E. B., and Nolan, M. O. Preliminary observations on the effect of sewage treatment processes on the ova and cysts of intestinal parasites. Sewage Works, **14**, 1274–1280 (1942).

TEMPERATURE RESISTANCE OF GOLDFISH MAINTAINED UNDER CONTROLLED PHOTOPERIODS[1]

WILLIAM S. HOAR AND G. BETH ROBERTSON

Abstract

Goldfish maintained under controlled photoperiods for 6 weeks or longer were relatively more resistant to a sudden elevation in temperature when the daily photoperiods had been long (16 hours) and relatively more resistant to sudden chilling when they had been short (8 hours). The magnitude of the effect varied with the season. Thyroid activity was slightly greater in fish maintained under the shorter photoperiods. The longer photoperiods stimulated more rapid growth of ovaries during late winter and early spring. The endocrine system is considered a link in the chain of events regulating seasonal variations in resistance to sudden temperature change.

Introduction

In latitudes where seasonal changes are marked, physiological cycles are common and prepare animals for such activities as reproduction and growth during the most favorable seasons. The constant annual cycle in the changing length of daily photoperiod seems to provide a reliable environmental regulator in many cases. Preliminary studies of the mechanism of regulating seasonally changing temperature resistance in goldfish suggested that this was another example of photoperiod regulation (6, 7). Groups of goldfish, maintained under otherwise identical conditions, were relatively more resistant to cold when kept under daily illumination of 8 hours' duration, alternating with 16 hours of darkness, than they were when maintained under the reverse conditions of illumination. On the other hand, the 16-hour-day fish were relatively more resistant to heat. Such a mechanism would seem to have high survival value in temperate and arctic regions. Otherwise, if fish were entirely dependent upon temperature acclimatization for their protection against seasonal temperature extremes, unusually abrupt temperature changes in the autumn or spring might cause serious mortality.

The initial tests were made during November and December (7). A second group of experiments, carried out during May and June of the following year, failed to substantiate the earlier findings. In fact, the results were frequently in the reverse direction and it was evident that the problem was more complex than the first experiments had suggested.

In an attempt to explain these contradictory findings, different groups of goldfish were placed under controlled photoperiods at intervals of about one month throughout an entire year. Resistance to both heat and cold was tested after 40 days or longer. Size differences in the samples, sex ratios, thyroid activity, and gonad development were also studied as possible factors responsible for the variability.

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Contribution from the Department of Zoology, The University of British Columbia, Vancouver, British Columbia, with financial assistance from the National Research Council of Canada.

Materials and Methods

Goldfish (*Carassius auratus*) were obtained from the Goldfish Supply Company, Stouffville, Ontario. They were maintained in a large indoor tank (10° to 15° C), under variable laboratory conditions of illumination, until placed under controlled lighting (about 60 ft-c at the water surface) at a temperature of 20° ± 1° C. The diet (about 0.4 g per fish per day) was a mixture of approximately equal parts of Pablum-mixed cereal (Mead Johnson & Co.) and Clark's commercial trout food, "Grower Crumbles".[2] The mean weights of the fish used in the tests, together with sex ratios and dates of the various experiments, are given in Tables I and II.

Groups of 65 goldfish were placed under each of the controlled photoperiods (8 hours of illumination alternating with 16 hours of darkness or 16 hours of illumination alternating with 8 hours of darkness) at intervals as indicated in Table II. At the end of the period of controlled illumination, samples of 20 or 21 fish from each photoperiod group were tested for cold resistance and an equal number tested for heat resistance. Another 20 fish, from the original 65, were used for the thyroid study.

The method of measuring temperature resistance has been described (6, 13). In brief, it involves a sudden transfer from the holding temperature of 20° C to a cold bath at about 2° C (range for entire series 2.0° to 5.8° C) or to a heat bath of about 36° C (range 34.7° to 36.4° C). The chill bath is cooled gradually during the next 1 to 2 hours until it reaches about 1° C and maintained at that temperature for the remainder of the test. Likewise, the temperature of the heat bath is raised and maintained between 37° and 38° C. Although the temperature of the lethal bath may vary slightly from test to test or may change during the test, it is emphasized that the fish which are being compared are always subjected to identical conditions of temperature. The different groups were distinctively fin-marked (day prior to the test) and placed at the same time in the lethal bath.

When the fish no longer respond to a standard mechanical stimulus (6), they are removed from the bath and placed in water at 20° C. After 1 hour, their condition (dead or alive), weight, length, and sex are recorded and the fish preserved in formalin. At a later date the animals are again weighed and the weight of the gonads also determined.

Thyroid activity was studied by measuring the uptake of a tracer dose (2 to 4 μc) of radioiodine, I^{131}. At intervals of 6, 12, 24, and 48 hours after injection, samples of 3 to 5 fish were killed, the thyroids removed, ashed in 2 *N* NaOH, samples diluted to volume, a 1 ml aliquot dried in a nickel planchet, and the activity expressed as per cent uptake of the administered dose.

Results

High Temperature Resistance

The results of the nine heat resistance tests are shown graphically in Fig. 1. The times plotted above or below the horizontal line are differences

[2]We are indebted to Mr. J. R. Clark of Salt Lake City, Utah, for contributions of trout food—presently being fed to all of our fish cultures.

between the times when various percentages of the 8-hour and the 16-hour fish were removed from the heat bath. The three bars in each series are the differences when 25%, 50% (black), and 75% of the fish in the samples had been removed. Values above the line are the lengths of time by which the 16-hour fish survived the 8-hour fish. Times below the line represent additional minutes of survival by the 8-hour animals.

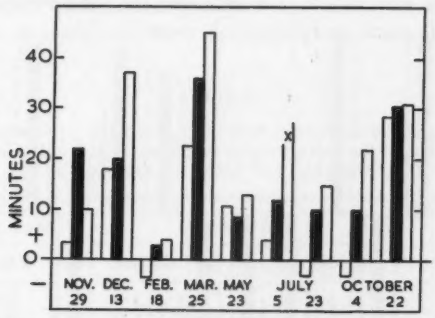

FIG. 1. Heat resistance of goldfish maintained under controlled photoperiods and tested on the dates indicated. Values show the difference between the survival times of the 16-hour and 8-hour groups; above the horizontal line, increased survival by the 16-hour fish; three bars in each series are differences in survival times when 25%, 50% (black), and 75% of the samples have been removed from the bath; X, level of mortality never reached by the more resistant group.

At the 50% and the 75% mortality levels, 16-hour fish are consistently more resistant to heat than 8-hour fish and thus react like the summer goldfish (6). However, the magnitude of the difference in resistance, produced by the controlled photoperiod, shows considerable variation. With the exception of the February test, the relative resistance of the 16-hour group was generally much more marked during the fall and winter than it was during the late spring and summer. Several possible sources of error have been examined in an attempt to determine whether this variability is actually due to some seasonal change in the physiology of the fish or to a bias in the samples.

It has been shown previously that, in heat tests, larger goldfish regularly die before the smaller animals and female goldfish slightly sooner than males (13). The mean weights and sex ratios of the different samples are presented in Table I. These factors may have contributed to the variability of the results but cannot explain the extreme differences observed. Differences between the mean weights of the 8-hour and 16-hour fish were no greater at one season than at another. The sex ratios vary considerably but are as often in one direction as in the other and show no obvious correlation with the temperature resistance picture.

It seems likely that some basic difference in the physiology of the animals tested at different times, rather than bias in size or sex ratios, is responsible for the variable picture seen in the different tests. In general, fish appear to be less responsive to an experimental manipulation of the photoperiod during

the summer, when their natural resistance to elevated temperatures is known to be relatively high (6). Since photoperiods were only controlled for 2 or 3 months, the natural changes in illumination prior to the start of the experiment may have stimulated physiological mechanisms which could not be readily reversed. It is suggested that, during the late spring and summer, all fish have such a marked high temperature resistance that the photoperiod manipulation is less effective. It is assumed that the February test is inconsistent because of some unrecognized error or bias in the experimental procedures.

TABLE I

Size and sex ratios of goldfish used in the heat resistance tests. (Numbers in parentheses are the standard deviations of the mean weights in grams; total number in sample is the sum of the males and females in each group)

Date of test	Weight (g)		Sex ratio (males: females)	
	8-hour	16-hour	8-hour	16-hour
Nov. 29	14.6 (5.0)	15.4 (3.8)	8:13	14: 7
Dec. 13	15.5 (3.4)	15.6 (2.2)	5:15	14: 7
Feb. 18	16.3 (4.8)	15.9 (4.1)	11: 9	13: 7
Mar. 25	16.8 (2.0)	18.1 (4.3)	7:13	9:11
May 23	18.7 (3.4)	17.4 (4.7)	10:10	9:11
Jul. 23	15.2 (2.9)	16.5 (3.4)	14: 5	8:12
Oct. 4	7.6 (2.6)	8.9 (2.6)	10:10	11: 9
Oct. 22	9.1 (2.7)	9.5 (3.0)	11:10	7:12

Low Temperature Resistance

The data for low temperature resistance (Fig. 2) are presented in a similar manner to those discussed for heat resistance. In this case, however, the values above the horizontal line show increased resistance on the part of the 8-hour fish; those below the line are increased resistance of the 16-hour groups. It should also be noted that the scale of this graph is logarithmic and the survival times very much longer.

Of the 12 tests, 7 were in one direction and 5 in the other. The different reactions, moreover, are not grouped in the different seasons. The over-all picture is a variable one which, by itself, provides little support for the hypothesis of photoperiodically induced changes in cold resistance. It will be shown, however, that when these data are considered in relation to several other factors, the hypothesis remains tenable.

It is evident that four tests carried out between October 15 and December 15 showed consistent and marked increased cold resistance in the 8-hour fish. This is the season when such protection would be most beneficial to the fish, and these results are in accord with two other tests carried out during November and December of the previous year (7). Moreover, three tests between May and July—when such protection would be of little value in nature—showed a reversed picture. This is also in accord with four spring and summer tests of the previous year (12). These results suggest that controlled photoperiods will produce slight or inconsistent effects on cold

resistance during the late spring, summer, and early fall. This line of argument, however, does not explain why the 16-hour fish should be more resistant to cold during the summer. In addition, the results of the tests performed on February 18 and October 4 still appear inconsistent with those performed before or after these dates.

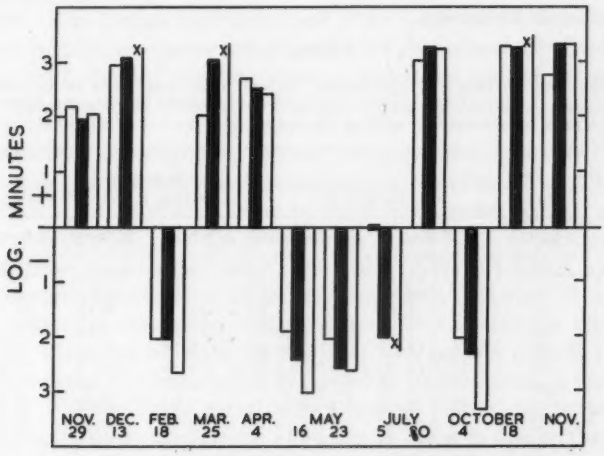

FIG. 2. Cold resistance of goldfish maintained under controlled photoperiods and tested on the dates indicated. Values show differences between the survival times of the 16-hour and 8-hour groups; above horizontal line, increased survival by the 8-hour group; below, increased survival by the 16-hour group; three bars in each series are differences in survival times when 25%, 50% (black), and 75% of the samples had been removed from the lethal bath; ×, level of mortality never reached by the more resistant group.

Reference to Table II fails to provide an explanation for these inconsistencies based on differences in the mean weights or sex ratios of the samples. It may be important, however, that the fish tested on October 4 were from a different stock than those used previously and were less than half the size of most of the samples used.

At least a partial explanation of these apparently different reactions is found in the difficulty of distinguishing "truly dead" from the "narcotized" fish in these cold tests. There is a marked tendency for the fish in the cold bath to show a cold narcosis. The degree of cold narcosis varies with the season and with the initial temperature shock experienced by the fish. As previously described, fish are removed from the bath when they no longer make a response to a standardized stimulus ("removals") and are then kept at 20° C to determine whether they recover ("narcotized" fish) or fail to do so ("truly dead" fish). Data presented in Table II show that the 8-hour fish tested on February 18 and October 4 showed a much greater tendency toward narcosis than those groups tested immediately before or after this date. Tendency towards narcosis was likewise marked in those summer groups where the 8-hour fish appeared to be relatively less resistant to cold. In

other words, the picture of low resistance to cold on the part of the 8-hour fish (reversal of the theoretical reaction) is at least partially due to the tendency of these fish to become deeply narcotized. It may be the entire explanation. If only those groups are considered in which the ratios of narcotized to dead fish were equal in the 8-hour and the 16-hour groups (samples of March 25, April 4, July 20, and October 18) a uniform picture of increased cold resistance by 8-hour fish remains.

TABLE II

Sizes, sex ratios, and the ratios of "narcotized" to one "truly dead" fish in the cold resistance tests. (Numbers in parentheses are the standard deviations of the mean weights in grams; sample size may be obtained by adding the male and female numbers in the sex ratios)

Date of test	Days controlled light	Weight (g)		Sex ratio (males: females)		Narcotized/dead	
		8-hour	16-hour	8-hour	16-hour	8-hour	16-hour
Nov. 29	40	17.7 (3.7)	17.2 (4.1)	9:12	10:11	0.6	1.1
Dec. 13	41	17.3 (4.4)	17.9 (4.2)	4:16	10:13	2.3	1.1
Feb. 18	42	15.5 (4.0)	16.7 (4.8)	12: 8	9:10	5.3	1.5
Mar. 25	35	17.4 (5.5)	19.3 (5.3)	10:10	7:13	0.6	0.9
Apr. 4	42	16.5 (4.9)	17.7 (3.8)	6:14	9:11	2.8	2.6
May 16	75	16.6 (3.9)	17.5 (3.6)	9: 9	12: 8	17.0	3.5
May 23	42	15.6 (4.3)	16.4 (3.5)	9:11	7:13	3.5	2.6
Jul. 5	49	16.7 (4.0)	16.0 (4.1)	7:12	8:12	5.7	3.0
Jul. 20	77	16.0 (3.6)	15.2 (3.4)	13: 7	10:10	0.5	0.8
Oct. 4	65	7.6 (2.6)	8.1 (2.7)	10:10	9:11	3.0	1.6
Oct. 18	77	10.3 (2.9)	10.0 (3.9)	14: 8	7:13	0.5	0.9
Nov. 1	90	13.6 (4.7)	10.7 (2.5)	9: 5	5: 7	0.6	1.8

It may be suggested that a consistent picture should be obtained if the data are plotted on the basis of "truly dead" fish. This, however, is impossible since the size of some of the samples is so greatly reduced that the curves are quite meaningless. For example, on May 16 there was only one and on July 5 only three of the "truly dead" fish in the 8-hour samples.

This tendency to show cold narcosis is a characteristic of (naturally produced) winter fish. In an earlier analysis of data for winter fish, procured in a strictly comparable manner, it was found that 89.5% of the fish from samples totalling 524 goldfish tested during the summer were "truly dead", while only 26.3% of samples totalling 354 fish tested during the winter were "truly dead" when removed from the cold bath (6).

In the present experiments, the relatively high proportion of narcotized 8-hour fish produces an apparent low cold-resistance picture since they are removed relatively early in the test. Actually, in all tests, the 8-hour fish showed either an increased resistance to cold death, as measured by the "removal times", or a tendency to show cold narcosis. Our basic knowledge of cold death, cold narcosis, and the interrelationships of the two phenomena is still too meager to carry the analysis further at this stage. It seems safe to conclude, however, that, as in the case of heat resistance, cold resistance of goldfish can be modified by control of the photoperiod but that the nature and the magnitude of the response show seasonal variation.

Some Other Changes Induced by Controlled Photoperiods

Although this study was primarily concerned with the comparisons of temperature resistance, two other effects were studied in a more general way. Thyroid function was examined because of the recognized seasonal changes in the thyroid activity of several fish and because of some rather contradictory data on the relationship of temperature acclimatization and thyroid function of fish (10). The gonads were examined as an index for photoperiod stimulation of the endocrine system associated with reproduction. The literature concerning both thyroid function and the endocrine control of reproduction has been reviewed (8, 9, 16).

Thyroid Activity

The goldfish thyroid was found to accumulate between 2% and 3.5% of the injected dose of radioiodine. Other workers have found similar, rather low, values for the goldfish (2). In general, the short-day fish accumulate more radioiodine than the long-day fish (Table III). The variability, as shown by standard deviations, however, is marked and there is scanty statistical evidence for a distinct separation of the groups. The t value for the difference between the means of all data for the first two sample periods is 1.27 (76 degrees of freedom)—corresponding to a $p > 0.1$. Limitations in the technique used make it inadvisable to carry the analysis of these data further. There are two potential sources of error. The first of these is due to the technique as applied to fish thyroids. The teleost gland is diffusely scattered in the throat region and variable amounts of the associated tissues, such as muscle and bone, are inevitably dissected out with the thyroid. With the Geiger counting technique used to evaluate the samples, an experimental error is expected due to the different amounts of self-absorption produced by the variable quantity of tissue associated with the thyroid. The second source of error is due to the presence of variable amounts of renal thyroid in the

TABLE III

Percentage uptake of radioiodine by goldfish thyroid at intervals after injection of tracer dose. (Numbers in parentheses are standard deviations for the mean I^{131} uptake, based on eight different experiments carried out at approximately 6-week intervals during 1 year)

	Short-day fish		Long-day fish	
Hours after injection	Number in sample	Mean uptake	Number in sample	Mean uptake
Male goldfish				
6	8	2.63 (0.77)	8	2.14 (1.30)
12	8	2.88 (0.97)	10	2.50 (1.14)
24	17	2.02 (1.23)	12	1.77 (0.80)
48	23	2.39 (1.36)	20	2.11 (0.98)
Female goldfish				
6	16	3.19 (0.93)	16	3.30 (1.52)
12	16	3.00 (1.34)	14	2.69 (2.33)
24	15	3.48 (2.20)	19	2.57 (1.52)
48	16	2.70 (2.06)	20	2.56 (2.88)

goldfish. Chavin (2) reports that in 47% of his goldfish the renal (head kidney) thyroid was actually more active than the throat thyroid. Goldfish are, however, extremely variable in this connection. The throat thyroid was more active in 23% of the fish, the two regions were of equal activity in 20% of the animals, and the head kidney accumulated no radioiodine in 10% of the samples.

In spite of the limitations in this technique, the data presented in Table III show a definite order. In seven of the eight comparative values given, the thyroids of the short-day (8-hour) fish accumulated slightly more of the radioiodine. The differences, although small and failing to show statistical significance, may be meaningful. In a series of experiments reported elsewhere (10), it has been shown that pretreatment of goldfish with thyroxine or with thyrotropic hormone will increase their resistance to sudden chilling in experiments of the type reported here. The increased thyroid activity may be, in some way, responsible for the increased cold resistance of the 8-hour fish. Further tests of this hypothesis are, however, required.

Gonad Development

The ratio of gonad weight in mg to the body weight in g was used as an index of the state of gonad development. With the relatively small testes (100 to 400 mg), the differences between the ratios of the 8-hour and the 16-hour fish were small (five or less) and as often in one direction as in the other.

The ovarian weights ranged from 300 mg to 1200 mg in fish of 14 to 20 g. In the case of the female, considerable growth of the maturing ova is expected in the period of 6 weeks prior to spawning and the controlled photoperiod produced the expected effect in some groups of goldfish. The 8-hour and 16-hour samples tested on February 18, March 25, and April 4 showed differences in ovary/body weight ratios of 19.0, 13.7, and 35.7 respectively with the relatively larger gonads in the 16-hour photoperiod fish. However, in samples tested on May 16 and 23, and on July 20, October 4, and November 1 the differences were too small to be considered significant: 0.6, 3.2, 1.5, and 9.5 respectively with the larger gonads in the 8-hour fish. The results may indicate that there is a refractory period following spawning and that the increased illumination may only be expected to have an effect on the reproductive system after this refractory period, during the winter and early spring. The number of fish in any particular seasonal sample was small (10 to 20 fish) and these findings cannot, at the moment, be considered conclusive.

Discussion

This and previous articles (6) demonstrate seasonal variations in the ability of goldfish to resist abrupt temperature changes. This effect, moreover, is independent of temperature acclimatization and is evidently regulated through the changing seasonal length of the photoperiod. Several seasonally varying physiological phenomena have now been shown to be controlled in a

similar manner. The first of these phenomena studied was related to reproductive behavior (18), and the importance of producing the young at the most favorable season for their survival and growth is obvious. More recently, several phenomena, not directly related to reproduction, have been shown to be similarly controlled. For example, the salinity preference of sticklebacks (1), certain reactions of trout to water currents (15), and the metabolism of crabs (3) have been altered by a suitable control of the photoperiod. Physiological mechanisms which enable animals to resist environmental extremes may be expected to have a high survival value. Abrupt seasonal temperature changes are not uncommon in temperate latitudes. These may occur too quickly for effective temperature acclimatization, and an increased resistance, for even brief periods, may be highly significant.

The existence of the phenomenon and its biological significance require no further discussion. The controlling mechanisms are not so easily explained. In fact, it will probably be impossible to give an adequate explanation until the basic physiology of heat and cold death is understood. The various theories have been often stated but, as yet, there is no entirely satisfactory explanation (5, 17).

In the present study, it seems probable that the endocrine system forms a link in the chain of regulatory events. The secretory activity of the pituitary gland will be regulated by light and this gland in turn will control such hormone-producing tissues as the thyroid gland. Seasonal variations in the cytology of the pituitary of the goldfish have been described (19). The demonstration that thyroid treatment modifies the temperature resistance (10) is suggestive but does not explain the basic physiological mechanisms. Unfortunately, we know too little about the action of thyroid hormone in fish or its action at the cellular level in any animal.

The basis of temperature resistance at the cellular level is highly speculative. Temperature-independent seasonal changes in oxygen consumption of fish (20) and in isolated fish tissues such as gills (4) have been described. One prominent theory of temperature death has to do with tissue anoxia (5) and this might be the actual point of regulation. On the other hand, the regulation may be, at least in part, through the lipid metabolism. In a series of articles, it has been demonstrated that temperature resistance of goldfish can be modified by dietary supplements of such lipids as lecithin and cholesterol (13) and that the tissue lipids change during temperature acclimatization (11, 14). Unpublished data (12) show that total tissue cholesterol and phospholipid are higher in the short-day fish and in the male fish (except during the breeding season). Both short-day fish and male fish are more resistant to cold (6, 7). Tissue-lipid changes associated with photoperiod control were not found to be statistically significant but may be an indication of what is happening in some particular tissue which is responsible for the variable temperature resistance. In any case, it seems likely that the physiological regulation is by the pituitary acting directly or indirectly through such glands as the thyroid and interrenal on some aspect

or aspects of tissue metabolism. Further studies of the basic mechanisms of temperature death are required before the sequence of regulatory events will be clear.

References

1. BAGGERMAN, B. An experimental study of the timing of breeding and migration in the three-spined stickleback (*Gasterosteus aculeatus* L.). Arch. néerl. zool. **12**, 105–318 (1957).
2. CHAVIN, W. Thyroid distribution and function in the goldfish, *Carassius auratus* L. J. Exptl. Zool. **133**, 259–279 (1956).
3. DEHNEL, P. A. Effect of photoperiod on the oxygen consumption of two species of intertidal crabs. Nature, **181**, 1415–1417 (1958).
4. EKBERG, D. R. Respiration in tissues of goldfish adapted to high and low temperatures. Biol. Bull. **114**, 308–316 (1958).
5. HEILBRUNN, L. V. An outline of general physiology. 3rd ed. W. B. Saunders Co., Philadelphia. 1952.
6. HOAR, W. S. Seasonal variations in the resistance of goldfish to temperature. Trans. Roy. Soc. Can. V, Ser. 3, **49**, 25–34 (1955).
7. HOAR, W. S. Photoperiodism and thermal resistance of goldfish. Nature, **178**, 364–365 (1956).
8. HOAR, W. S. Endocrine organs. *In* The physiology of fishes, Chap. 6, Vol. 1. *Edited by* M. E. Brown. Academic Press, New York. 1957.
9. HOAR, W. S. The gonads and reproduction. *In* The physiology of fishes, Chap. 7, Vol. 1. *Edited by* M. E. Brown. Academic Press, New York. 1957.
10. HOAR, W. S. Endocrine factors in the ecological adaptation of fish. *In* Endocrinology. *Edited by* A. Gorbman. John Wiley & Sons, New York. 1959.
11. HOAR, W. S. and COTTLE, M. K. Some effects of temperature acclimatization on the chemical constitution of goldfish tissues. Can. J. Zool. **30**, 49–54 (1952).
12. HOLLANDS, M. The effects of photoperiod on the goldfish (*Carassius auratus*). M.A. Thesis, University of British Columbia. 1956. Unpublished.
13. IRVINE, D. G., NEWMAN, K., and HOAR, W. S. Effects of dietary phospholipid and cholesterol on the temperature resistance of goldfish. Can. J. Zool. **35**, 691–709 (1957).
14. MUSACCHIA, X. J. and CLARK, M. R. Effects of elevated temperatures on tissue chemistry of the Arctic sculpin *Myoxocephalus quadricornis*. Physiol. Zool. **30**, 12–17 (1958).
15. NORTHCOTE, T. G. Effect of photoperiodism on response of juvenile trout to water currents. Nature, **181**, 1283–1284 (1958).
16. PICKFORD, G. E. and ATZ, J. W. The physiology of the pituitary gland. New York Zoological Society, New York. 1957.
17. PRECHT, H., CHRISTOPHERSEN, J., and HENSEL, H. Temperatur und Leben. Springer-Verlag, Berlin. 1955.
18. ROWAN, W. Light and seasonal reproduction in animals. Biol. Revs. **13**, 374–402 (1938).
19. SCRUGGS, W. M. The epithelial components and their seasonal changes in the pituitary gland of the carp (*Cyprinus carpio*, L.) and goldfish (*Carassius auratus*, L.). J. Morphol. **88**, 441–469 (1951).
20. WELLS, N. A. Variations in the respiratory metabolism of the Pacific killifish *Fundulus parvipinnis* due to size, season, and continued constant temperature. Physiol. Zool. **8**, 318–336 (1935).

LIFE HISTORY AND ECOLOGY OF HOROGENES CHRYSOSTICTOS GMELIN (HYMENOPTERA, ICHNEUMONIDAE), A PARASITE OF EPHESTIA SERICARIUM SCOTT (LEPIDOPTERA, PHYCITIDAE)[1]

RODERICK C. FISHER[2]

Abstract

Horogenes chrysostictos Gmelin, a common parasite of lepidopterous larvae, was reared in the laboratory in larvae of *Ephestia cautella* Walk., *E. elutella* Walk., *E. sericarium* Scott, *Hofmannophila pseudospretella* Staint., *Endrosis sarcitrella* L., *Galleria mellonella* L., and *Achroia grisella* Fab. The size and coloration of the adult and period of preimaginal development are regulated by the size and species of the host and environmental temperature. The duration of the egg stage and of the five larval instars are recorded over the viable temperature range. Characters are given which distinguish the first and fifth larval instars from those of *Nemeritis canescens* Grav., also a parasite of *E. sericarium*, and the rates of development of both species are compared over the range 15° to 30° C. *H. chrysostictos* develops more quickly at all temperatures. Its sex ratio is usually 3 males : 2 females; the reproductive potential is highest at 25° C with a mean of 39.4 progeny per female. The adult behavior in mating, host finding, and oviposition is described. Two modes of combat occur between supernumerary larvae in competition for the possession of their host: fighting with the mandibles between young first instar larvae, and combat by humoral inhibition which occurs between young first instar larvae and those in later instars. In both cases the older parasite survives competition.

Introduction

The ichneumonids *Horogenes chrysostictos* Gmelin and *Nemeritis canescens* Grav. (Ophioninae, Campoplegides), which parasitized the Mediterranean flour moth *Ephestia sericarium* Scott (Lepidoptera, Phycitidae), were selected for a study of the phenomenon of multiparasitism. The biology of the latter parasite species is already well known (Diamond (6), Beling (3), Ahmad (2)). General information on the biology of *H. chrysostictos* and descriptions of its larval stages compared with those of *N. canescens* are presented here as the necessary preliminaries to the study of multiparasitism. Since *H. chrysostictos* can easily be bred on *E. sericarium* in the laboratory, this account of its biology is presented for those who may wish to use it for experimental purposes.

Distribution and Host List

H. chrysostictos is widely distributed in Europe and Asia. It is known as a parasite of many species of Lepidoptera, but chiefly of *Plutella maculipennis* Curt., *Hyponomeuta padella* L., and *Ephestia elutella* Walk. A list of the recorded hosts is given in Table I. It is probably of wide occurrence in the United States and Canada, for it is synonymous with the North American

[1]Manuscript received April 18, 1959.
Contribution from Department of Zoology, University of Cambridge, Cambridge, England.
[2]Postdoctoral Fellow, National Research Council of Canada. Present address: Entomology Laboratory, Belleville, Ontario.

species *H. kiehtani* Viereck (Walley, personal communication). As far as is known, *H. chrysostictos* has not previously been reported as a parasite of *Ephestia sericarium*, though records of attack on this species by *Horogenes* spp. appear in the literature (Adair (1), Beling (4), Walley (34)).

My own experiments confirmed that *H. chrysostictos* will attack and develop normally in the following species of Lepidoptera: Phycitidae, *Ephestia sericarium* Scott, *E. cautella* Walk., *E. elutella* Walk.; Galleriidae, *Achroia grisella* Fab.; Oecophoridae, *Hofmannophila pseudospretella* Staint., *Endrosis sarcitrella* L. In these species *H. chrysostictos* elicits no host blood reaction and completes development in 21 to 23 days at 25° C. It has also been recorded from *Galleria mellonella* L. in Europe (Smits van Burgst (25), Thorpe (29)). However, though it would oviposit in this host, its development in an English strain of *G. mellonella* maintained at Cambridge was arrested in the second instar, whereas the same parasite stock would complete development in 28 to 30 days at 25° C in Canadian *G. mellonella* kept in culture at Belleville, Ontario.

TABLE I

Insects recorded as hosts of *H. chrysostictos* in the literature

Family	Species	Country	Authority
Lepidoptera			
Galleriidae	*Galleria mellonella* L.	Holland	Smits van Burgst (25)
		Britain	Thorpe (29)
Glyphiterygidae	*Anthophila fabriciana* L.	Germany	Schutz and Roman (22)
	Chloreuthis myllerana F.	Germany	Schutz and Roman (22)
Gracilariidae	*Gelechia interruptella* Hb.	Germany	Schutz and Roman (22)
	Gracilaria syringella F.	Britain	Morley and Rait Smith (17)
Heliodinidae	*Heliodines roesella* F.	Germany	Schutz and Roman (22)
Hyponomeutidae	*Argyresthia nitidella* F.	Britain	Morley and Rait Smith (17)
	Hyponomeuta cognatellus Hb.	Britain	Morley and Rait Smith (17)
	H. euonymellus L.	Britain	Morley and Rait Smith (17)
	H. malinellus Zell.	Germany	von Wahl (33)
		Russia	Meier (14), Ruzaev (20), Telenga (26)
	H. padellus L.	Britain	Morley and Rait Smith (17)
	Plutella maculipennis Curt.	Germany	Torka (30)
		Russia	Romanova (19), Meier (13)
Nymphalidae	*Vanessa urticae* L.	Britain	Morley and Rait Smith (17)
Oecophoridae	*Agonopteryx cyanivora* Meyr.	Morocco	Kozlovsky and Rungs (10)
	Endrosis sarcitrella L.	Britain	Morley and Rait Smith (17)
	E. lactella Schiff.	Britain	Morley and Rait Smith (17)
	Hofmannophila pseudospretella Staint.	Britain	Richards (18)
Olethreutidae	*Rhyacionia buoliana* Schiff.	Germany	Habermehl (8)
		Britain	Thorpe (27)
Phalaenidae	*Lycophotia porphyrea* Schiff.	Germany	Wagner (32)
Psychidae	*Fumea* sp.	Britain	Ratzburg in Morley (16)
	F. casta Pall.	Britain	Morley and Rait Smith (17)
	F. intermediella Briand d'Uzelle	Britain	Morley (16)
Pterophoridae	*Oidaematophorus* sp.	Germany	Habermehl (8)
Phycitidae	*Ephestia elutella* Walk.	Belgium	Leclercq (11)
		Britain	Richards (18)
Pyralidae	*Zophodia convolutella* Hb.	France	Seyrig (23)
Pyraustidae	*Loxostege sticticalis* L.	Russia	Meier (15), Sacharov (21)
Tineidae	*Talaeporia pectinella* (*sic*)	France	Giraud in Morley (16)
	Tinea sp.	Britain	Morley (16)
Tortricidae	*Archips murinana* Hb.	Czechoslovakia	Kolubajiv (9)
	Tortrix resinana (*sic*)	Britain	Ratzburg in Morley (16)
Hymenoptera			
Tenthredinidae	*Lophyrus* sp.	France	Gaulle in Morley (16)
	Nematus sp.	Britain	Ratzburg in Morley (16)
	N. gallicola Steph.	Britain	Thorpe (28)
	N. salicis Ashm.	France	Dours in Morley (16)

NOTE: Owing to taxonomic confusion in the genus *Horogenes*, the records for hosts of this species are open to doubt. Morley (16) considers that all *Hyponomeuta* records are for *H. majalis* Grav.

In surveys of 15 flour and provender mills in Norfolk and Suffolk, England, *H. chrysostictos* was found in 9 mills with *E. sericarium*, *Endrosis sarcitrella*, and *H. pseudospretella*. In only one was a large population found, breeding on *E. sericarium* (percentage parasitism: 12% of 250 hosts dissected). *N. canescens* was also taken from 10 mills on the same host. In the four mills in which both species occurred together neither was present in large numbers. It appears that the species usually occur separately, but through the movement of infested flour sacks and colonization by the adults they must frequently be brought into contact in the same mill.

The Adult

The adult of *H. chrysostictos* has been described by Morley (16), and Richards (18) has compared it with *N. canescens*. Adults of both sexes are mainly black, the mouth parts are pale yellow, and the legs yellowish to reddish brown. The tibiae of the mid and hind legs are darkened proximally and distally so that they have a banded appearance. The adult is subject to variations both in size and color which are regulated by the temperature at which it is bred and on the size of its host. Females bred at 18° to 20° C from mature larvae of *E. sericarium* are usually 6.5 to 7.0 mm long and have light reddish-brown legs; males reared under the same conditions are 6.0 to 6.5 mm long and have legs which are mainly yellow. The adults that emerged from mature *E. sericarium* larvae and from *E. elutella* were usually about 5.0 mm, and occasionally only 4.0 mm, long. Adults bred at 25° and 28° C are lighter in color than those bred at lower temperatures and have pale yellowish-red legs with uniformly pale hind tibiae.

Preimaginal Development

Egg

The eggs are laid within the body cavity of the host and at first float freely in the haemolymph. The backward direction of flow of the haemolymph carries them to the posterior part of the haemocoele where they often become attached to the Malpighian tubules. The translucent white eggs are of the hymenopteriform type and are variable in size, but those laid by females 6.0 to 7.5 mm long are commonly about 0.25 mm long and 0.05 mm wide at the broadest part (ranges 0.32×0.065 mm and 0.249×0.05 mm). The incubation period varies with the environmental temperature (Table III). The structural changes during development at 25° C are shown in Fig. 1, A. By 40 hours body segmentation is complete, and the head capsule is fully differentiated by 50 hours. The eggs generally hatch in 59±2 hours at 25° C, by which time they are, on average, 0.492×0.128 mm in size.

A few hours before eclosion the first instar larva begins to move within the chorion and just before the larva is hatched the pharynx begins to contract. In the last 5 minutes the larva swells rapidly, apparently by drinking the fluid surrounding it; this swelling is presumed to assist the dorsal longitudinal splitting of the chorion as the larva hatches (Fig. 1, A: 6 and 7).

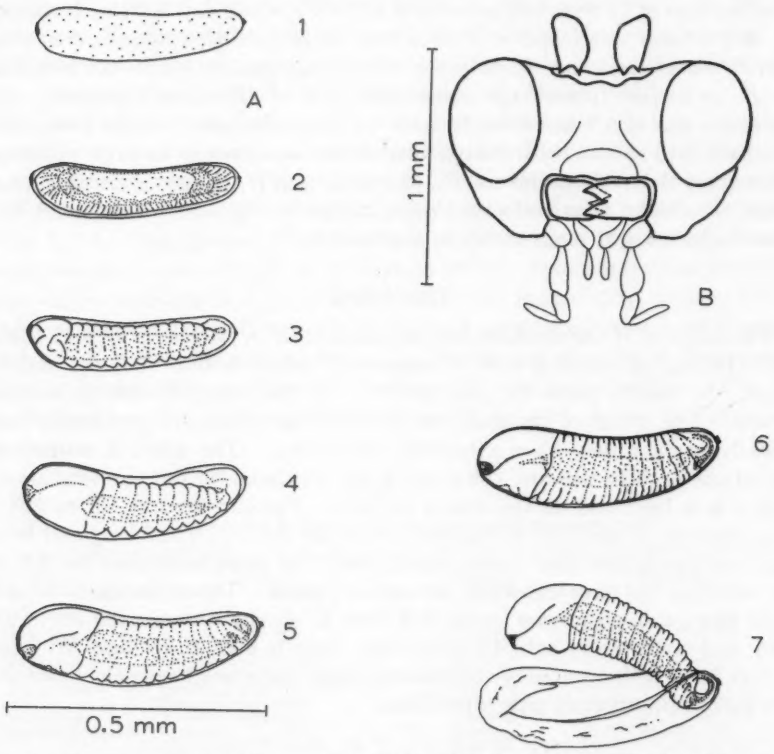

FIG. 1. *H. chrysostictos*: A, egg development at 25° C—1, newly laid; 2, 20 hours; 3, 40 hours; 4, 50 hours; 5, 58 hours; 6, 58–59 hours: embryo swells before 7, eclosion; B, ventral view of head.

Larval Stages

The first instar larva lives freely in the haemocoele of the host and is of the caudate type. It has a yellowish-brown, sclerotized head with well-developed mandibles, and a slender body of 13 segments and a caudal appendage. It increases in size from 0.65 mm long and 0.10 mm wide at eclosion to 1.75 × 0.45 mm at maturity. The intermediate dimensions of larvae reared at 25° C are shown in Table II.

Both the head capsule and the mouth parts (Fig. 2) are similar to those described for the Ophionines by Cushman (5), Tothill (31), Meier (12), and Thorpe (28). The mandibles (*md*) are 0.022 to 0.025 mm in length (measured along the anterior edge) with a roughly triangular base and a strong falcate spine. Immediately posterior to them and within the food meatus (*fm*) there are two minute sensoria which are thought to be rudiments of the labial palpi (*lplp*). The surface of the head is smooth, bearing only a ring of eight small setae round the mouth parts. The terminology used in the description of the larval head capsule and facial structures follows that of Short (24).

TABLE II

Dimensions (in mm) of the head capsule, mandible, and body of larvae of *H. chrysostictos* reared at 25° C

	Body			Head capsule		
	Length (+ tail)	Width	Tail length	Length	Width	Mandible
1st instar						
At eclosion						
59 hours	0.65	0.10	0.165	0.133	0.095	0.025
72 hours	0.942	0.10	0.203	0.133	0.10	0.025
120 hours	1.26	0.28	0.275	0.133	0.11	0.025
At ecdysis						
Day 6	1.75	0.45	0.33	0.134	0.112	0.025
2nd instar						
Day 6	1.75	0.3	0.25	0.232	0.301	0.068
Day 7	2.6	0.35				
3rd instar	2.8	0.68	0.21			0.08
Day 8	3.6	0.9				
4th instar	4.0	1.3				0.092
Day 9	5.5	1.5				
5th instar						
Day 10	6.5	1.5				0.102
Day 12	7–7.5	1.5–2.0				
Prepupa	7–7.5	1.8–2.0				
Day 12–13						
Pupa						
Day 14	6–6.5	1.5–2.0				

The closed tracheal system is composed of two lateral tracheal trunks that extend along the body just above the lateral line with accessory lateral commissures below the main trunks in the second and third body segments. The trunks branch dorsally and ventrally into each segment and bifurcate in the caudal appendage into two smaller tracheae with accessory branches.

The disposition of the internal organs is the same as that described for Ophioninae by Tothill (31) and Meier (13). The mid-gut is a simple tube that becomes enlarged with food to fill the body cavity. During larval life it does not open into the dilated hind-gut and no defaecation takes place until the last instar larva emerges from the mature resting larva of *E. sericarium* immediately prior to pupation.

The first instar larva of *H. chrysostictos* can readily be distinguished from that of *N. canescens* by differences in the head capsule. In *H. chrysostictos* the head capsule is distinctly longer than broad (0.133 mm long × 0.10 mm wide) and its ventral edge is emarginated behind the mouth and before the posterior apex of the plate, thus giving it a distinctly two-lobed appearance (Fig. 3, A). In *N. canescens* the head capsule is slightly broader than long (0.116 mm long × 0.138 mm wide) (Fig. 3, B), with the ventral edge only slightly and uniformly concave, so that it has only a single lobe at the posterior apex of the lateral plate. When the first instar larvae are distended with

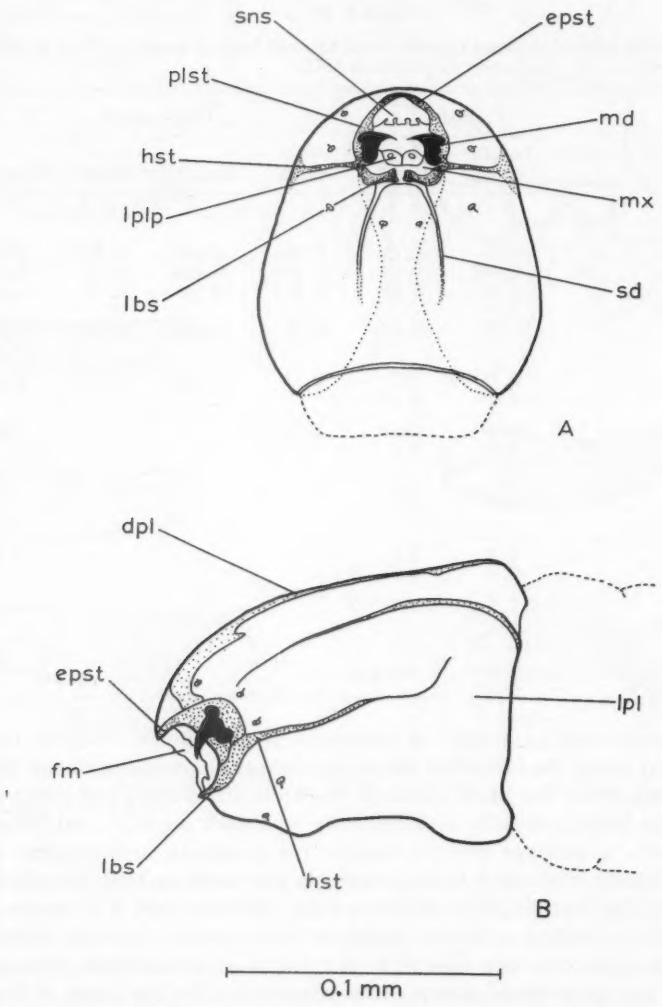

FIG. 2.* *H. chrysostictos:* head capsule of first instar larva—A, ventral view; B, lateral view.

*Abbreviations used in the figures:

ant—antennal organ
clp—clypeus
dpl—dorsal plate of head capsule
epst—epistoma
fm—food meatus
hst—hypostoma
lbs—labial sclerite
lpl—lateral plate of head capsule
lplp—labial palp
md—mandible
mplp—maxillary palp
mx—maxilla
ol—ocular line
plbs—prelabial sclerites
plst—pleurostoma
sd—salivary duct
sns—sensoria
ss—stipital sclerite

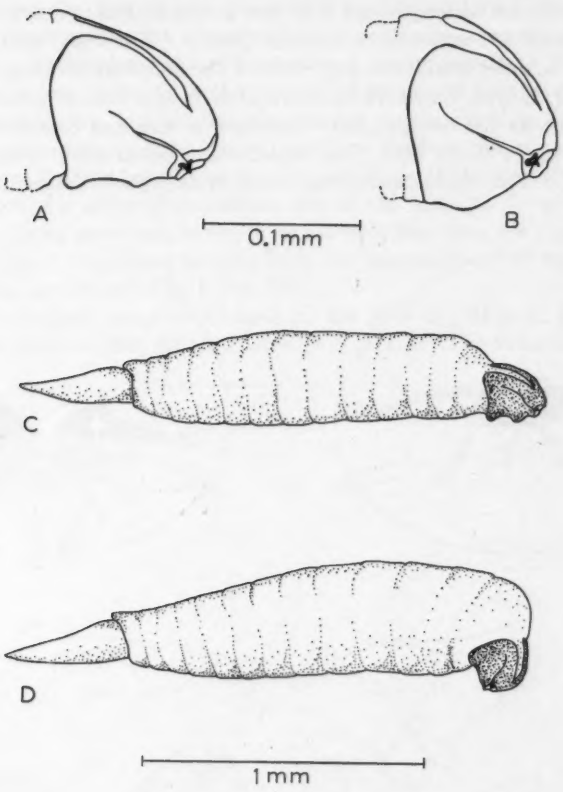

FIG. 3. Diagnostic features of first instar larvae: head capsules—A, *H. chrysostictos;* B, *N. canescens;* lateral view of larvae—C, *H. chrysostictos;* D, *N. canescens.*

ingested food, the head capsule of *H. chrysostictos* is usually forced forwards (Fig. 3, C) whereas that of *N. canescens* is moved to a more ventral position (Fig. 3, D). The brain of the second instar larva of *N. canescens* therefore lies above the head capsule, rather than behind it as in *H. chrysostictos.* At the end of the first instar, when the body is fully distended, *N. canescens* is curved whereas *H. chrysostictos* is usually straight.

The succeeding four larval instars are readily distinguished from the first by their lack of a well-defined, sclerotized head capsule. The caudal appendage becomes progressively smaller in the second and third instars and is absent from the fourth and final instars. The body changes in shape from the caudate type of the first instar through the second instar, which is intermediary, to the turgid, fusiform shape of the subsequent instars.

The second instar (Fig. 6, A) lasts for about two days at 25° C and grows to about 2.6 mm in length and 0.35 mm in width. The head capsule measures

0.232 to 0.235 mm in length and 0.30 mm in width, but is not heavily sclerotized and does not maintain a constant shape. The only sclerotized parts of the body are the mandibles and some of the facial struts (Fig. 4, A).

The third, fourth, and fifth instars follow in the 8th, 9th, and 10th days after eclosion as the larva rapidly increases in size and completely destroys the host (Fig. 6, B, C, D). The final dimensions of each instar are shown in Table II. The caudal appendage remains as a pointed termination to the

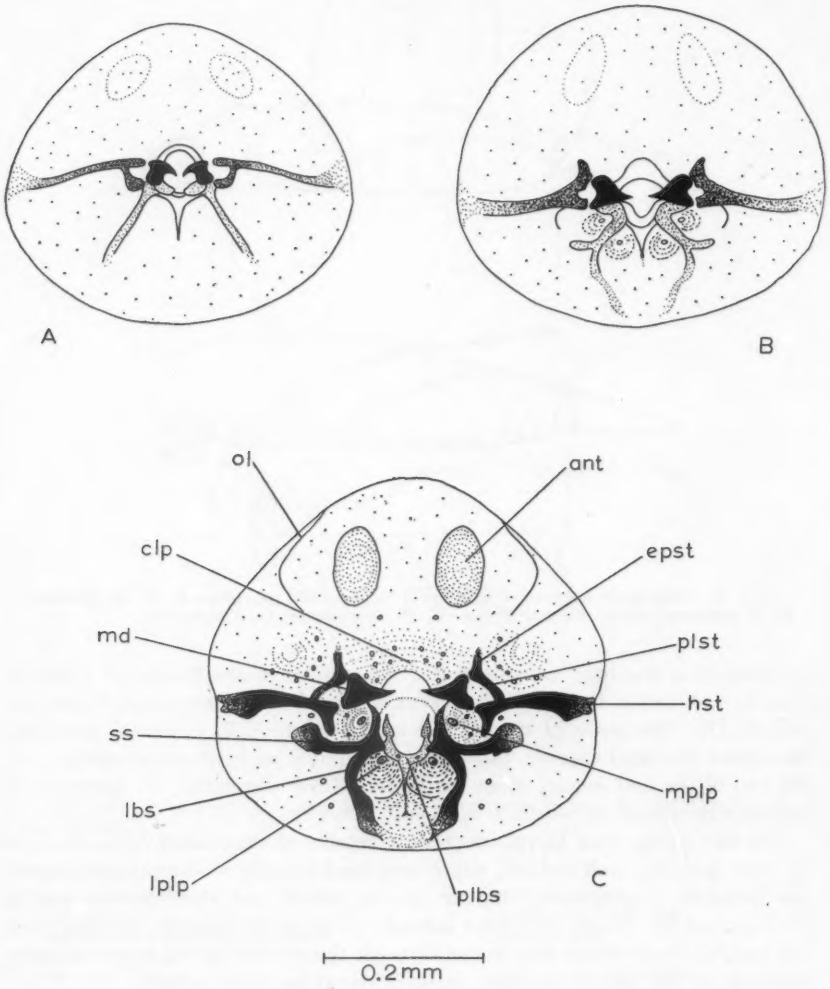

FIG. 4. *H. chrysostictos:* head and facial structures of larva—A, second instar; B, fourth instar; C, fifth instar.

body and is largely covered by a posteriorly projecting dorsal lobe of the 13th segment. These instars are creamy white in color and have no pigmentation except on the mandibles and facial sclerites. In the second to fourth instars the facial skeleton conforms to the typical ichneumonid pattern but only the epistoma, hypostoma, and stipital sclerites are pigmented (Fig. 4, A, B).

Though these instars can be distinguished by their shapes, the most reliable means of identification found was the size and shape of the mandibles (Fig. 5). Measurement of a series of mandibles (tip of the spine to the anterior basal articulation) from mounted larval skins showed that they are classed in four distinct groups. The mean lengths from the measurement of 10 individuals in each instar are recorded in Table II.

At 25° C the final instar is attained on the 10th day (Fig. 6, D). In this the facial skeleton is fully differentiated (Fig. 4, C). The ventral portions of

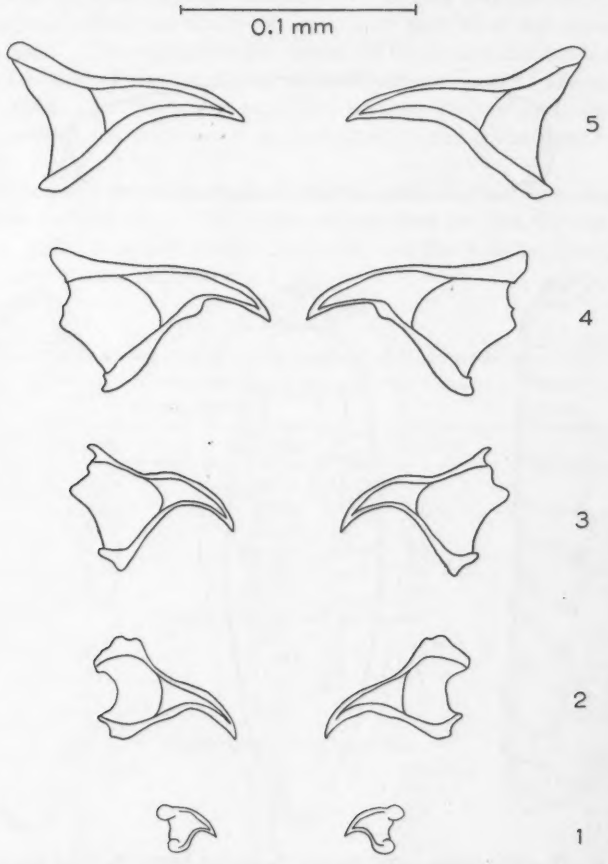

FIG. 5. *H. chrysostictos:* mandibles of first to fifth instar larvae.

the labial sclerites are only faintly sclerotized so that the labial ring appears to consist of two semicircles joined dorsally and open below. This differs from *N. canescens* which has a more complete heart-shaped ring. The epistomal ring is incomplete but is visible on either side as a bar extending dorsally. In *N. canescens* it is completely unpigmented but in *H. chrysostictos* it is recognizable as a small darkly sclerotized peg projecting dorsally from the pleurostoma.

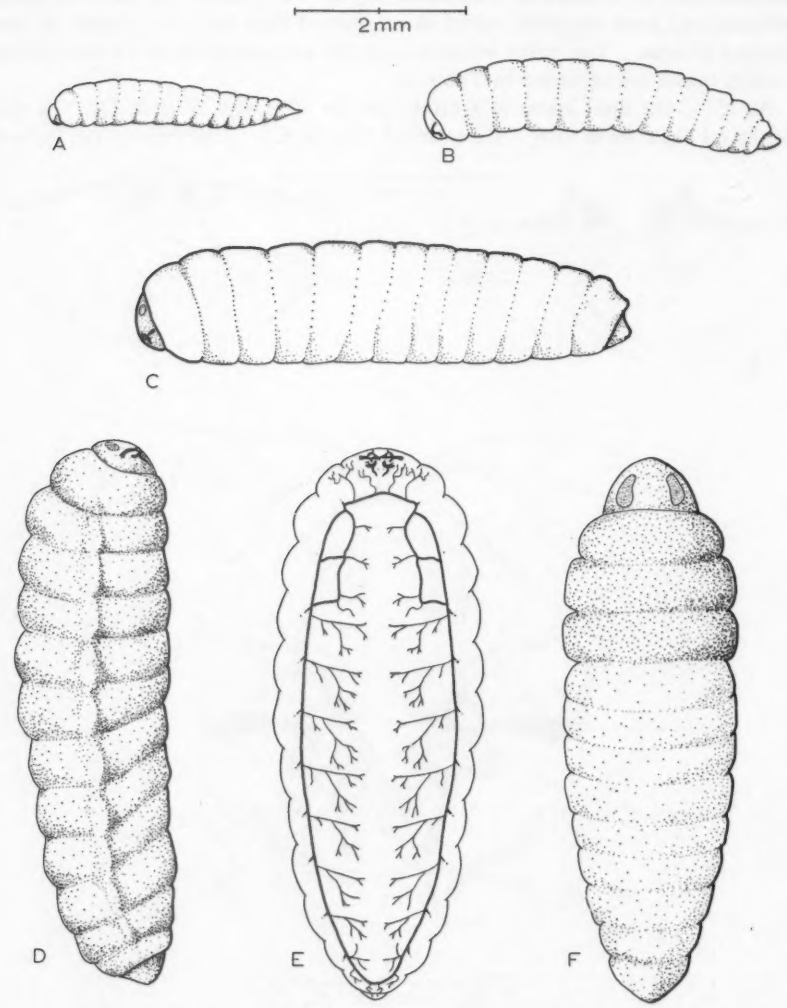

FIG. 6. *H. chrysostictos:* larval stages—A, second instar; B, third instar; C, fourth instar; D, fifth instar, lateral view; E, fifth instar tracheal system, ventral view; F, prepupa.

The cuticle of the head and body bears no setae except round the facial skeleton, and is folded into minute densely packed papillae. On the central surface of the pro-, meso-, and meta-thoracic segments there are three pairs of smooth, faintly sclerotized spots, 0.15 mm in diameter, which mark the position of the legs.

The tracheal system conforms to the typical ichneumonid arrangement (Fig. 6, E). There are nine pairs of spiracles all open to the exterior, the first on the posterior margin of the prothoracic segment, the remainder on the anterior margins of the first eight abdominal segments. The two lateral tracheal trunks are joined by transverse commissures in the second and third segments.

Pupation and Emergence

On the 12th or 13th day the mature larva breaks through the dried host skin and spins a cylindrical white cocoon, darkly banded at the center, within that of the host. The prepupal stage is marked by the appearance of darkened areas on each side of the head that indicate the position of the developing compound eyes. The thoracic segments become dilated and smooth and there is a marked constriction of the body behind the metathoracic segment (Fig. 6, F).

The exarate pupa develops rapidly from the 14th day and the adults begin to emerge on the 21st day. The males emerge from the 21st day to the 25th day, with a peak emergence on the 22nd day, and the females from the 22nd to the 26th day with a peak on the 23rd day.

TABLE III

Effect of temperature on egg and larval development of *H. chrysostictos* and *N. canescens*

Temp., °C	*H. chrysostictos*		*N. canescens*	
	Time	*X*/time	Time	*X*/time
	Duration of egg stage*			
15°	168 hr	5.952	230 hr	4.347
18°	118	8.475	171	5.848
25°	59	16.95	69	14.49
28°	48	20.83	52	19.23
30°	45	22.22	50	20.0
	Duration of egg and 1st instar†			
15°	20 hr	5.0	28 hr	3.571
18°	13	7.692	19	5.262
25°	7	14.28	7	14.28
28°	6	16.67	6	16.67
30°	6	16.67	6	16.67
	Duration of preimaginal life†			
15°	49 days	2.041	102 days	0.980
18°	36	2.777	56	1.786
25°	22	4.545	23	4.345
28°	20	5.0	22	4.545
30°	20	5.0	21	4.762

*X = 1000.
†X = 100.

Effect of Temperature on Development

No information on the duration of the life cycle was found in the literature apart from Beling's (4) record for *Angitia armillata*. She bred this species from *E. sericarium* in less than 4 weeks at 18°–20° C and commented that the life cycle was shorter than that of *N. canescens*. Observations on the duration of the egg, first larval instar, and complete preimaginal period made at constant temperatures of 15°, 18°, 25°, 28°, and 30° C confirmed that this is also true of *H. chrysostictos*. Like *N. canescens*, it cannot complete its development at 10° or 33° C.

The durations of these stages for both species are shown in Table III. Though the rates of development of each species increase similarly with temperature, *H. chrysostictos* develops more quickly than *N. canescens* throughout the viable temperature range, particularly at lower temperatures.

General Biology

On emergence in flour mills, the adults are positively phototactic, flying to the windows and occasionally into the open where they visit flowers, presumably to obtain food. After mating and feeding, the females return to contaminated flour in search of their hosts.

Mating and Sex Ratio

H. chrysostictos is an arrhenotokous species. The preoviposition period is short since mating occurs immediately after emergence and the females are ready to lay shortly afterwards. Virgin females readily oviposit soon after emergence if host larvae are available. The mating behavior is similar to that described by Beling (4) for *A. armillata*. On perceiving a female at a distance of up to 5 cm, the male raises his abdomen and begins a rapid vertical vibration of the wings and antennae. He approaches the female with a hesitant, jerky gait and, if accepted, rapidly flexes the abdomen beneath the thorax, extending the genital armature to clasp the female. Copulation lasts from 1 to 20 minutes during which both insects remain motionless. It terminates when the female resumes activity and drags the attached male behind her until he releases her abdomen. Mating occurs frequently, especially when the adults are crowded in a stock container.

The sex ratio is very variable; in general males are more numerous than females. In laboratory stocks the proportion of female progeny varied from 0 to 58% depending on the age of the parents when mating, their population density, and the density of their hosts. The spermatozoa of the males rapidly attain maturity after emergence, and this is followed by reproductive senescence. Most males contain no active spermatozoa by the fifth or sixth day after emergence at 20° C. However, females accumulate eggs in the oviducts so that when hosts are available large numbers of unfertilized eggs are laid, thus producing male progeny. The same result is observed when the host–parasite ratio is high.

Since Flanders (7) found that multiple mating in *Macrocentrus ancylivorus* Roh. (Braconidae) prevented fertilization by malposition of premature ejection of the spermatophore, *H. chrysostictos* females were isolated on emergence and mated once with 2-day-old males. Routine stocks were set up with three females and 30 hosts each. Over 12 generations the sex ratio of the progeny was approximately three males to two females. The proportion of females in samples taken from a flour mill in Suffolk varied from 40% to 60% and the totals of these throughout the year gave a mean sex ratio of three females to two males.

Host Finding and Oviposition Behavior

H. chrysostictos is strongly attracted to the odor of flour and oatmeal but it has little precise guidance to the position of its host. This odor stimulates the parasite to alight on the flour; the female then moves slowly over the surface, running forward in short bursts of activity, while palpating it with the tips of the antennae. When found, flour contaminated with the odor of the hosts is subjected to intense antennal investigation and to stabbing with the ovipositor. Stabbing is made at random in respect of individual hosts and continues for a long period if no hosts are encountered with the ovipositor. When a host is found the parasite stabs at it repeatedly until the cuticle is penetrated. At this the host recoils violently and this movement is an important stimulus to oviposition. The final stimulus to oviposition is probably the chemical condition of the host perceived by sense organs at the tip of the ovipositor, for, as will be shown in a future paper, the parasite is able to distinguish between healthy hosts and those already parasitized by its own or another species. Oviposition can generally be recognized by the further elevation of the abdomen and sudden contraction of the abdominal pleurae; afterwards the ovipositor is quickly withdrawn and, after examining the host once more, the parasite runs aside to clean the antennae, legs, and ovipositor. The duration of oviposition varies from 2 or 3 seconds to more than a minute, depending on the time taken to penetrate the host's cuticle.

Longevity

In the absence of food isolated males live for 2 days and females for 3 days at 25° C. At 20° C males live 4 to 8 days, females 4 to 7 days; at 15° C males live 8 to 9 days and females 9 to 10 days. The longevity of both sexes fed on split raisins and allowed to drink water is increased to 10 to 14 days on the average at 25° C and up to 60 days at 10° C.

Fecundity and Reproductive Potential

The ovaries and oviducts of freshly emerged females contain 200 to 230 eggs; but since the eggs are progressively produced during adult life the actual numbers laid may be greater than this. However, the number of progeny per female is very much smaller, because many hosts are superparasitized. The fecundity of the species was estimated by presenting a newly emerged mated female with 50 hosts each day until death at 20° C (Fig. 7). The

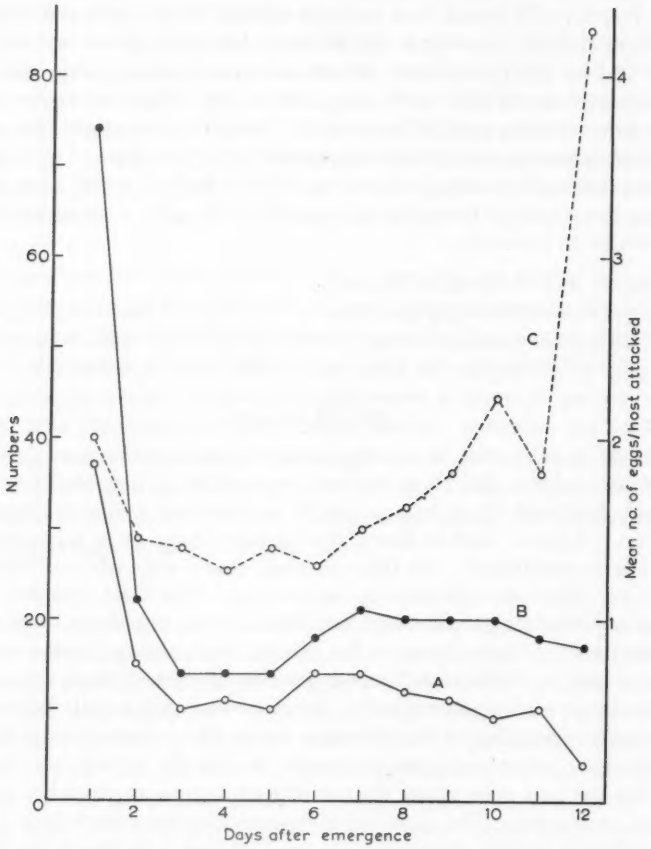

FIG. 7. Fecundity of *H. chrysostictos* at 20° C when given 50 hosts per day: A, number of hosts attacked; B, number of eggs laid; C, mean number of eggs laid per host attacked.

TABLE IV

Progeny of 25 females each with 100 hosts at constant temperatures in °C

	15°	18°	25°	28°	30°
	8	34	35	34	31
	5	50	41	31	20
	8	24	43	49	16
	3	36	48	28	44
	4	41	30	32	27
Mean	5.6	37.0	39.4	34.8	27.6
Maximum	8	50	48	49	44

number of hosts attacked and of eggs laid in them were counted daily. Usually a female lays one egg at each attack, but at high host densities and towards the end of life her restraint on superparasitism breaks down, so that some hosts are heavily superparasitized (Fig. 7, C). In view of this the actual numbers of progeny at a given temperature are the only reliable indication of the reproductive potential. The number of progeny per female at different constant temperatures is shown in Table IV. The percentage parasitism at all temperatures is very variable but it is greatest at 18° and 25° C. Yet the parasitism achieved when *H. chrysostictos* is exposed to a single set of larvae until death at 18° C (37.0%) is not significantly different from that obtained by daily presentation of fresh hosts (38.2%). The reduction in reproductive potential at 15° C is thought to be due to inhibition of the insect's movement by lower temperature rather than to a smaller egg production.

Superparasitism

H. chrysostictos is able to discriminate between parasitized and healthy hosts and partially to avoid ovipositing in the former. However, as in *N. canescens* this restraint breaks down under conditions of high parasite–host ratio and high host density in a limited area, and superparasitism occurs. When this happens competition between the supernumerary parasite larvae takes place, as only one can mature in each host.

On hatching from the egg, the young larvae move about actively in the haemocoele of the host by vigorous movements of the abdomen and tail.

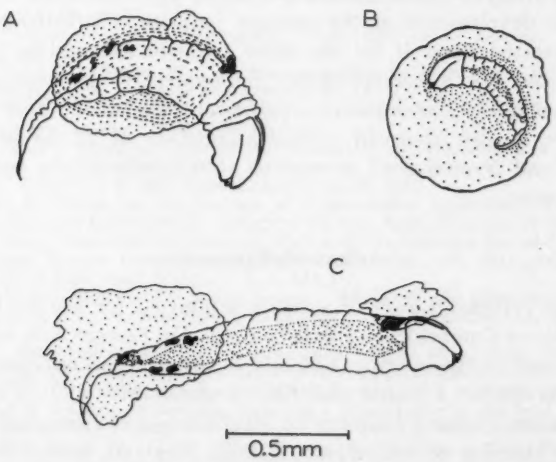

FIG. 8. Elimination of supernumerary larvae of *H. chrysostictos:* by physical combat—A at 2 days, C at 4 days with melanization and partial encystment; by physiological inhibition with complete encapsulation—B.

When two larvae meet, one embeds its mandibles in the body of the other and remains in this position from several minutes to half an hour. After they have separated there may be a leakage of body fluid from the punctured cuticle and in some cases the whole larva becomes considerably shrunken. Following this the wounded larva becomes susceptible to the blood reactions of the host, encystment by haemocytes, and deposition of melanin on the cuticle. Parasites that have been eliminated in this way show patches of melanin in one or more places on the cuticle (Fig. 8, A) that contain pairs of distinctly darker spots. The distance between the members of each pair corresponds to the distance between the mandibles of the successful parasite. In larvae dissected 4 days after oviposition there was an aggregation of host haemocytes around the wound, and by 6 days this had enlarged considerably and had either enclosed the whole parasite in a loose cyst or had formed a partial cyst around it.

Normally the supernumerary larvae are eliminated within 24 hours of hatching from the egg, but on some occasions the parasites do not meet until the second day of larval life; then the biting reaction is the same and similar host reactions of melanin deposition and phagocytosis follows, although the phagocyte cyst does not grow over all of the now enlarged larva (Fig. 8, C). When the two larvae separate, the victor resumes its active life while the victim hardly moves at all. The reactions of melanization and encystment follow, sealing off the parasite from the internal environment of the host and killing it.

A second means of elimination occurs when *H. chrysostictos* superparasitizes a host already containing a parasite in the late first instar or subsequent instars. The development of the younger parasite is arrested by a form of inhibition, exerted upon it by the older parasite and acting through the medium of the host's haemolymph. Subsequently the younger parasite dies and is completely enclosed in a spherical cyst by the host (Fig. 8, B). This reaction is also observed in multiparasitism of *E. sericarium* by *H. chrysostictos* and *N. canescens*, in relation to which it will be fully discussed in a future paper.

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References

1. ADAIR, E. W. A preliminary list of insects associated with cotton in Egypt. Agr. J. Egypt, **8**, 80–88 (1918). (Abstract *in* Rev. Appl. Entomol. A, **7**, 164.)
2. AHMAD, T. The influence of ecological factors on the Mediterranean flour moth, *Ephestia*, and its parasite *Nemeritis canescens*. J. Animal Ecol. **5**, 67–93 (1936).
3. BELING, J. Zur Biologie von *Nemeritis canescens*. I. Züchtungserfahrungen und ökologische Beobachtungen. Z. angew. Entomol. **19**, 223–249 (1932).
4. BELING, J. Zur Biologie und Zucht der Schlupfwespe *Angitia armillata* Grav. Arb. biol. Reichsanstalt Land- u. Forstwirtsch, Berlin-Dahlem, **20**, 237–244 (1933).
5. CUSHMAN, R. A. *Thersilochus conotracheli* Riley, a parasite of the plum curculio. J. Agr. Research, **6**, 847–855 (1916).
6. DIAMOND, V. R. The biology of *Nemeritis canescens*, a parasite of the Mediterranean flour moth. Rept. Entomol. Soc. Ontario, **60**, 84–89 (1929).
7. FLANDERS, S. E. The role of the spermatophore in the mass propagation of *Macrocentrus ancylivorus* (Braconidae). J. Econ. Entomol. **38**, 323–327 (1945).
8. HABERMEHL, H. Beiträge zur Kenntnis der palaearktischen Ichneumonidenfauna. Konowia, **1**, Pts. 1–2; **3**, pts. 4–5; **6**, 77–86, 97–112, 234–240, and 266–282 (1922). (Abstract *in* Rev. Appl. Entomol. A, **11**, 365.)
9. KOLUBAJIV, S. Die Ergebnisse der Züchtung von parasitischen Insektarten aus ihren Wirten in der Staatlichen Versuchsanstalt in Prag (In Czechoslovakian.) Acta Soc. Entomol. Czechoslov. **31**, 59–68; 113–120; 155–163 (1929–1933). (Abstract *in* Rev. Appl. Entomol. A, **23**, 229.)
10. KOZLOVSKY, S. and RUNGS, C. Note sur *Depressoria cynarivora* (Lep.-Oecophoridae), ravageur du *Cynara scolymus* au Maroc. Bull. soc. sci. nat. Maroc, **12**, 101–103 (1933). (Abstract *in* Rev. Appl. Entomol. A, **21**, 399.)
11. LECLERCQ, J. Insects brought with hay from meadows into the hay-loft. Entomol. Mon. Mag. **82**, 138 (1946).
12. MEIER, N. F. On the morphology of some parasites of the family Ichneumonidae. Rept. Bur. Appl. Entomol. **2**, 25–39 (1922). (In Russian.) (Abstract *in* Rev. Appl. Entomol. A, **11**, 304.)
13. MEIER, N. F. Biologie von *Angitia fenestralis* Holmgr. der Parasiten von *Plutella maculipennis* Curt. und einige Worte über Immunität der Insekten. Z. angew. Entomol. **12**, 139–152 (1926).
14. MEIER, N. F. Parasites (Ichneumonidae and Braconidae) bred in Russia from injurious insects during 1881–1926. (In Russian.) Rept. Bur. Appl. Entomol. **3**, 75–91 (1927). (Abstract *in* Rev. Appl. Entomol. A, **16**, 200.)
15. MEIER, N. F. Parasitic Hymenoptera reared from *Loxostege sticticalis* in the U.S.S.R. in 1929. Rept. Bur. Appl. Entomol. **4**, 499–501 (1930). (Abstract *in* Rev. Appl. Entomol. A, **19**, 285.)
16. MORLEY, C. Ichneumonologia Britannica. 5 vols, 1903–1914. London. Vol. 5. 1914. pp. 175 and 193.
17. MORLEY, C. and RAIT SMITH, W. The hymenopterous parasites of the British Lepidoptera. Trans. Roy. Entomol. Soc. London, **81**, 133–183 (1933).
18. RICHARDS, O. W. Ichneumonid parasites of warehouses and flour mills. Proc. Roy. Entomol. Soc. London, B, **18**, 19–24 (1949).
19. ROMANOVA, V. P. Parasites (fam. Ichneumonidae) of injurious insects in the Northern Caucasus. (In Russian.) Bull. N. Caucasus Plant Protect. Sta. **6–7**, 133–138 (1929). (Abstract *in* Rev. Appl. Entomol. A, **19**, 563.)
20. RUZAEV, K. S. Notes on the biology of *Hyponomeuta malinellus*. Plant Protect. (U.S.S.R.), **6**, 213–219 (1929). (Abstract *in* Rev. Appl. Entomol. A, **19**, 288.)
21. SACHAROV, N. L. Report of the Entomol. Station of the Astrakan Soc. of Fruit Growing Gardening, Market Gardening and Field Cultivation. 3A, 1912, Astrakan, 1913. (Abstract *in* Rev. Appl. Entomol. A, **1**, 535.)
22. SCHUTZ, K. T. and ROMAN, A. Schlupfwespen. (A list of 270 ichneumonids and braconids bred from various insects in Saxony.) Isis Budissina, **12**, 1–12 Bentzen (1931).
23. SEYRIG, A. Observations sur les Ichneumonides. (1ier. série.) Ann. Soc. Entomol. France, **95**, 157–172 (1926). (Abstract *in* Rev. Appl. Entomol. A, **15**, 104.)
24. SHORT, J. R. T. Morphology of head of larval Hymenoptera with special reference to the head of the Ichneumonoidea, including a classification of the final instar larvae of the Braconidae. Trans. Roy. Entomol. Soc. London, **103**, 27–66 (1952).
25. SMITS VAN BURGST, C. A. Twee Parasiten van de Wasmot (*Galleria mellonella* L.). Entomol. Ber. Ned Vereen, **6**, 259–298 (1924). (Abstract *in* Rev. Appl. Entomol. A, **12**, 513.)
26. TELENGA, N. A. Hymenopterous parasites (Ichneumonidae) reared at Kuban in 1927. Plant Protect. (U.S.S.R.), **6**, 225–226 (1929). (Abstract *in* Rev. Appl. Entomol. A, **19**, 289.)

27. THORPE, W. H. Parasites of the Pine Shoot Moth *Rhyacionia buoliana*. Bull. Entomol. Research, **21**, 387–412 (1930).
28. THORPE, W. H. Notes on the natural control of *Coleophora laricella* the larch case-bearer. Bull. Entomol. Research **24**, 273–277 (1933).
29. THORPE, W. H. Mating between males of *Angitia chrysosticta* and females of *Nemeritis canescens*. Proc. Roy. Entomol. Soc. London A, **13**, 58–59 (1938).
30. TORKA, V. Parasiten der Kohlschabe (*Plutella maculipennis* Curt.). Ans. Schädlingskunde, **5**, 39 (1929). (Abstract *in* Rev. Appl. Entomol. A, **17**, 462.)
31. TOTHILL, J. D. The natural control of the fall webworm, (*Hyphantria cunea* Drury) in Canada. Can. Dept. Agr. Tech. Bull. **3**, 1–107 (1922).
32. WAGNER, A. C. W. Schlupfwespen and ihre Wirte: Zuchtergebnisse von Hamburger Entomologen. Verh. Ver. Naturw. Unterhaltung, **20**, (1928), reprint Hamburg (1929). (Abstract *in* Rev. Appl. Entomol. A, **19**, 127.)
33. VON WAHL, C. Die Gespinstmotten (*Hyponomeuta malinellus* und *H. variabilis*). Hauptstelle für Pflanzenschutz in Baden an der Grossh. landw. Versuchsanstalt. Augustenberg. Flugblatt Nr. 5. Z. landwirtsch. Versuchsw. Deut-Oesterr. **22**, 235 (1919). (Abstract *in* Rev. Appl. Entomol. A, **8**, 288.)
34. WALLEY, G. S. New parasitic Hymenoptera. Can. Entomologist, **51**, 190–194 (1929).

THE ENERGETICS OF HARBOR SEALS IN AIR AND IN WATER WITH SPECIAL CONSIDERATION OF SEASONAL CHANGES[1]

J. S. HART AND L. IRVING[2]
With the assistance of B. MACKENZIE

Abstract

The energetics of six harbor seals in air at temperatures from 25° C to −20° C and in water from 25° C to 0° C was studied in animals acclimatized to summer conditions at Woods Hole, Mass. Over the above temperature range, the temperature of skin of the back cooled from about 35° to 1° with lowering temperature of the medium. Temperatures of the flippers were less dependent upon the surroundings than those of the body skin. The dependence of metabolism upon temperature was operative below 2° C in air and below 20° C in water (the critical temperatures), but the body skin temperature at these critical temperatures was the same, viz. 21° C. Metabolism in air and in water was the same over the range of comparable skin temperatures. Body insulation was equal in air and in water at the respective critical temperatures. The insulation index of the air was approximately 20 times that of the water. Heat conductivity of living blubber to water averaged 2.5 cal/cm²/hr/°C, which exceeded that reported for dead blubber by about 50%.

When compared with seals tested at St. Andrews during December, summer seals had a higher critical temperature, a lower body insulation index at the critical temperature, and a warmer body skin temperature for the same metabolic rate. No seasonal changes were found in thermoneutral metabolic rate, and no discernible changes in skin temperatures or in thermal gradients in the blubber.

Introduction

The phenotypic modifications of mammals following changes in climate fall into two general categories, metabolic and insulative (4). In the former, cold is met by the development of an enhanced ability to produce heat, while in the latter it is met by reducing heat loss either by greater pelage insulation or greater cooling of peripheral tissues. Natural metabolic acclimatization, which has been observed by Hart in deer mice (5), may occur in species of small body size. In animals such as the fox and porcupine with thick fur, Irving and co-workers (9) have found that there was a marked restriction of the emission of heat associated with the thicker fur during winter. Their findings complemented the observations of Scholander *et al.* (10) on the importance of fur for the low critical temperatures of arctic animals during winter, and those of Hart (3) on the greater winter pelage insulation of the larger mammals.

In "bare skinned" animals such as pigs (6), and seals (7), fur has little or no protective value and the skin surface assumes a dominant role in the loss of heat. Any modifications to suit low winter air or water temperatures must therefore be brought about either by greater heat production or by reduction

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[2]Present address: Arctic Health Research Center, Anchorage, Alaska.

of heat loss through greater cooling of the living peripheral tissues. The remarkable capability of seals to show surface cooling in cold environments has already been demonstrated by Irving and Hart (7). The close correlation between the degree of cooling and the metabolic response in different individuals suggested the direction for further study of seasonal changes.

In the present investigation, the results obtained on a sample of harbor seals during December 1955 are compared with those obtained on a sample of the same population during the summer of 1956. With these additional data it has been possible to give a more detailed account of the energetics of seals in air and in water than was possible during the winter of 1955.

Experimental Animals

Six harbor seals, *Phoca vitulina concolor*, captured at Boothbay Harbour, Maine, in May 1956 were shipped to Woods Hole, Mass., during July 1956. They were held in a large pound and fed frozen fish during the period of captivity from July to September. Observations on the animals were made in August and September, during which period water temperature varied from 20 to 22° C. The animals were presumably from the same population as those studied during the winter of 1955 (7). The similarity of the animals and methods of testing facilitate direct comparison of the 1955 and 1956 results.

Methods

The test methods used were identical with those reported earlier (7) except for the automatic instead of manual recording of temperature gradients. The availability of a cold room also made it possible to utilize a greater range of air temperatures than was possible in 1955. In brief, the procedure consisted in the testing of one animal per day at three or four different temperature conditions in air and in water. Surface temperatures were measured on a potentiometer indicator and tissue temperatures at various depths were recorded at 16 points in each 4 minutes by means of gradient needles (7) prepared by L. J. Peyton.

After thermal balance was obtained in approximately 1 hour, three or four 10–15 minute samples of expired air were obtained for the measurement of oxygen consumption during the second hour, as previously described (see Fig. 3).

In addition to these measurements, a few observations were made on the heat flow from the seal's surface to water of various temperatures to correlate heat flow with the observed thermal gradients measured close to the same site. For this purpose the rise of temperature of the water in a small Dewar flask held against the seal's side was measured with a calibrated thermocouple and a sensitive Leeds and Northrup d-c. amplifier. The calorimeter, which was previously calibrated by producing heat electrically, had a precision of approximately $\pm 5\%$.

Results

Resting Metabolism

The metabolism of seals during the summer was dependent on environmental temperature below about 20° C in water and 2° C in air. The resting metabolism of all the seals in air and water warmer than these critical temperatures is shown in Table I together with body weights and respiratory quotients. The mean resting metabolism (18.3 ml/min/$kg^{3/4}$) did not differ significantly from that found for winter animals (Table I).

TABLE I

Average metabolism of seals at Woods Hole in air above 0° C and in water above 20° C

Seal No.	Weight, kg	O_2 consumption		R.Q.	No. of tests
		ml/min	ml/min/$kg^{3/4}$		
1	32.8	237	17.4	.76	13
2	31.7	219	16.3	.76	7
3	25.1	207	18.4	.69	7
4	24.4	217	19.8	.74	5
5	22.8	208	18.9	.71	7
6	27.4	213	18.1	.70	7
Mean summer	27.4		18.3±.38		
Mean winter*	33.7		19.3±.55		

*Excluding "runt" seal.

Owing to insufficient data in winter, it was not possible to compare the metabolic rates of summer and winter seals in air below the critical temperature but the comparisons for animals tested in water (Fig. 1) illustrate some appreciable differences. At subcritical temperatures, summer seals at Woods Hole had higher metabolic rates than winter seals at St. Andrews. The difference was reflected in a higher critical temperature in water in summer than in winter animals. In spite of the seasonal trends it may be seen that the exceptionally high metabolism of the "runt" seal tested during the winter was still greater than that found for any of the seals during the summer. The individual variability was also exemplified by summer seal No. 2 (◒), which had a much lower metabolism than any of the winter seals and which did not maintain a constant deep body temperature. The data on these exceptional seals have not been included in the subsequent results.

Skin Temperatures in Air and in Water

The data shown in Fig. 2 indicate that skin temperature on the surface of the back and on the flippers is closely dependendent on air or water temperature. In air, skin temperatures on the back fell markedly with lowering of the ambient temperature. The difference in temperature between skin and ambient increased with lowering tempearature even in the thermoneutral range (above 2° C). There were no discernible seasonal differences in skin temperatures, but insufficient data were obtained in air below the thermoneutral range for an adequate comparison.

The temperatures of the flippers in air, while following the general trend of temperatures of skin of the back, were more variable and this implies separate control of heat flow through these appendages as noted in our earlier study (7).

In water, skin surface temperatures showed a small increase above water temperatures as the latter fell from 26° to 0° C. As previously shown (7), differences in temperature between skin and water were small, the greatest

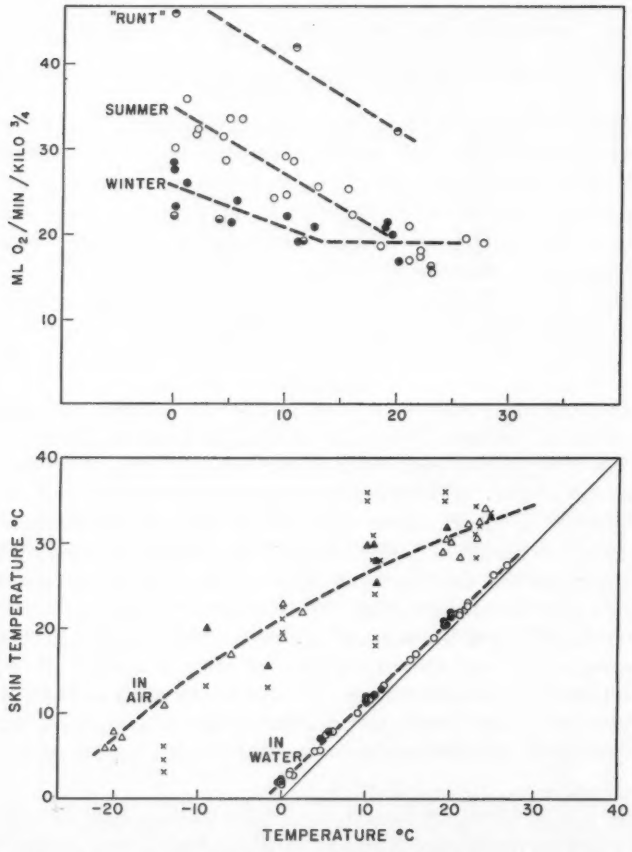

FIG. 1. Oxygen consumption of harbor seals during winter (●) and summer (○) in water. Large variation is illustrated by exceptionally high metabolism of "runt" (◓) seal (winter) and low metabolism of seal No. 2 (◒, summer).

FIG. 2. Body skin temperature of seals during winter (▲ ●) and summer (△ ○) are shown as a function of air (▲ △) and water (● ○) temperature. Each point is average of 3–5 measurements. Flipper temperatures (×) as a function of air temperature are more variable than those of body skin. Solid line indicates equality of skin and environmental temperature, broken lines indicate skin temperature trends. Difference between skin temperature and ambient is given by broken line minus solid line.

difference (2° C) occurring at 0° C. In water, no discernible seasonal differences in skin temperatures were evident but the accuracy of thermocouple measurements was probably insufficient for their detection.

Temperature Gradients and Heat Flow

Temperature gradients in the seal's tissues represent one parameter effecting heat flow which becomes meaningful when the conductivity of the tissues is known. Winter gradient curves, obtained from temperature measurements at various depths, have been previously reported (7). Figure 3 shows temperature contours for a summer seal under five different thermal conditions. The rapid attainment of thermal equilibrium within about 30 minutes indicates active regulation, which is also manifested in ice water by synchronous oscillations of 1 to 2° C in temperature of the subcutaneous tissues to a depth of 23 mm. In many such tests the flippers showed a marked tendency for independence in regulation, differing by 5 to 10° C from the temperature of the body surface.

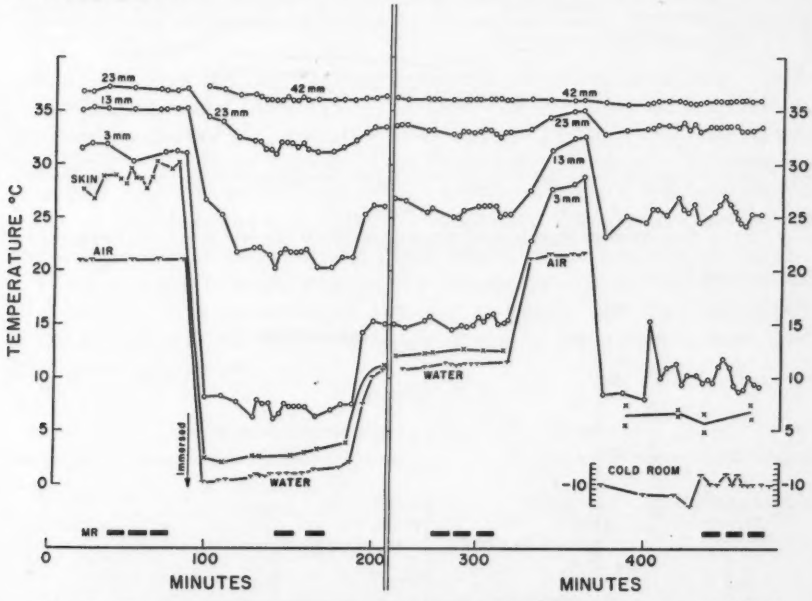

FIG. 3. Temperature measurements at various depths in a seal in air at 21° C, in water at 0–2° C, in water at 10–12° C, in air at 22° C, and in air at −10° C. Air temperatures, mean of five points within 25 cm of seal, agreeing within 1.5°. Water temperatures, mean of eight points in tank, agreeing within 0.1°. Skin temperature mean of two points on back just posterior to axilla and differing less than 0.5°. Horizontal bars marked "MR" show when gas samples taken.

In Fig. 4 are shown simultaneous records during 9 hours of two gradient needles inserted into the back 16 cm apart. The general similarity in response at the two locations and the speed of attainment of thermal balance is demonstrated. Plotting the simultaneously recorded gradients of the two positions

at time 560 (Fig. 5) showed that around 5 to 20 mm in depth the two curves differed by 2° to 3° C. Owing to the shifting of the needles by 1 to 3 mm during the 9 hours of recording, the difference of 2° to 3° C represented the error to be expected when the gradient is steep. In water at 5° C (Fig. 5) the base of the blubber was 3° C cooler than the maximum recorded temperature of the deep musculature. Thus the temperature gradient is not confined to blubber and may at times involve only part of it.

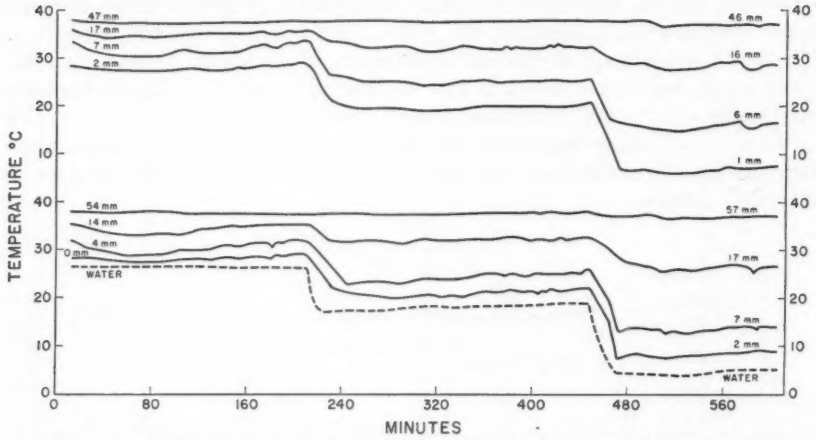

FIG. 4. Simultaneous records of temperature gradients of a seal at points separated by 16 cm on back over thorax. Upper record, point embedded in rib; lower, point intercostal.

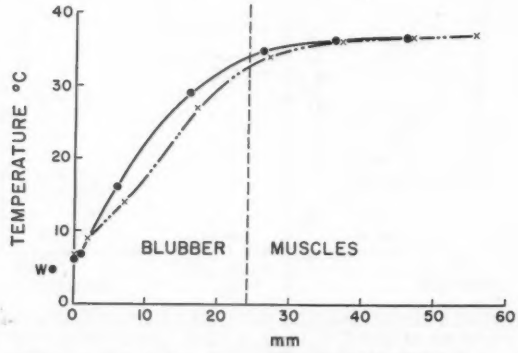

FIG. 5. Simultaneous recorded gradients from time 560 in Fig. 4.

To compare the temperature gradients during summer and winter, the temperature difference from skin to 37° C has been correlated with the depth of tissue corresponding to that difference (gradient length) shown in Fig. 6. Whether tested in air or water, summer or winter, the temperature–depth patterns were sensibly the same. The individual variability was marked and exceeded the errors in measurement.

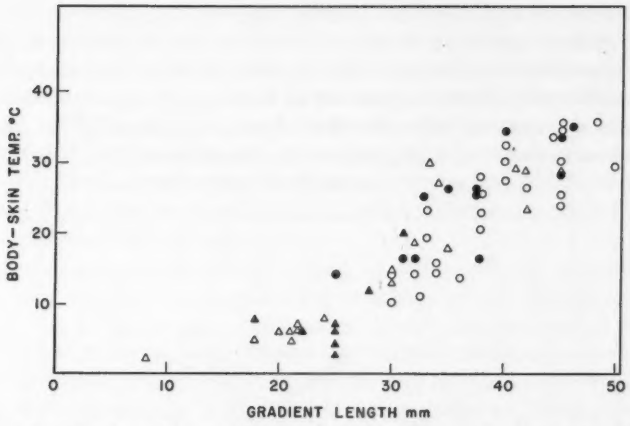

FIG. 6. Length of gradient as a function of the difference in temperature between deep body and skin surface for summer (△ ○) and winter (▲ ○) in air (▲ △) and in water (● ○)

Heat flow from the seal to water was measured on four animals at two or three temperatures (Table II). The heat flow increased with increasing gradient and decreasing temperature of the water. Conductivity values showed considerable variability between animals and temperatures. The average value of 2.5 cal/cm²/hr/°C is about 50% greater than the 1.6 cal/cm²/hr/°C measured by Scholander *et al.* (12) for dead blubber. The greater conductivity is attributable to the circulation of the living blubber.

Two seals (Nos. 1 and 3) were tested at temperatures well above the critical level (20° C). It is interesting to note that in warm water the conductivity of the blubber was not greater than that found at temperatures lower than the critical temperature.

TABLE II

Thermal characteristics of living seal blubber

Seal No.	Water temp., °C	Heat flow, cal/cm²/hr	Gradient,* °C/cm	Conductivity, cal/cm²/hr/°C
1	27.0	9.2	4.2	2.2
	18.0	15.8	7.0	2.3
	4.5	29.8	11.0	2.8
2	23.0	8.4	4.5	1.9
	11.0	12.1	6.5	1.9
3	26.0	11.2	4.0	2.8
	12.5	20.7	6.5	3.2
	2.0	31.6	9.5	3.3
6	22.0	13.9	6.0	2.3
	9.2	18.6	8.0	2.1
	0.0	29.8	11.7	2.5

*Measured in outer 10 mm of tissue.

Metabolism in Air and Water

The oxygen consumption of seals in air and water of various temperatures during the summer is shown in Fig. 7. As illustrated previously in Fig. 1, oxygen consumption began to increase in water at temperatures below 20° C and increased to nearly twice the thermoneutral rate at 0°C. In air, the oxygen consumption began to increase at temperatures just below 2° C and increased to about 50% above thermoneutral at −20° C.

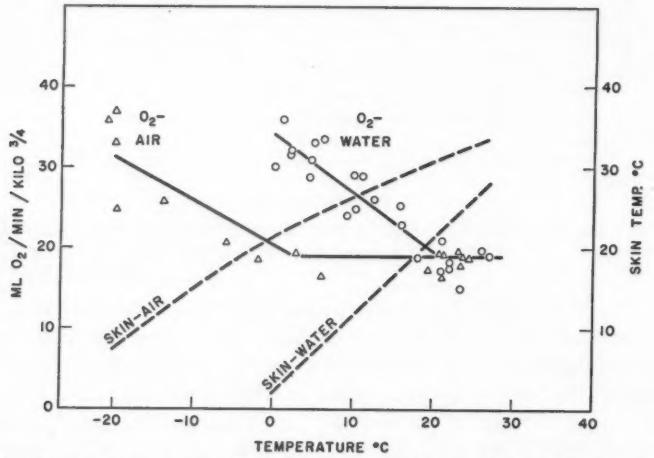

FIG. 7. Oxygen consumption of seals in air (△) and in water (○) at different temperatures during the summer. Body skin temperatures as a function of temperature of medium are also shown.

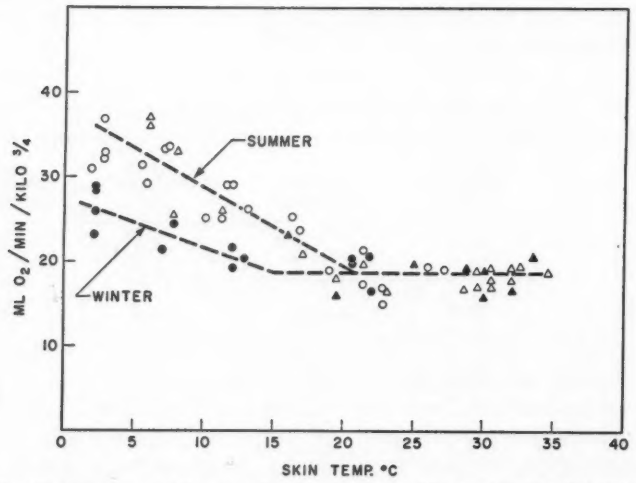

FIG. 8. Oxygen consumption as a function of body skin temperature for seals during winter and summer. Symbols same as for Fig. 2.

The broken lines in Fig. 7 give the average temperatures of skin of the back as a function of air or water temperature previously illustrated in detail in Fig. 2. A comparison of metabolism and skin temperatures brings out the fact that for a given skin temperature in air or water the metabolism is nearly the same. Thus, the critical temperature occurs at a skin temperature of approximately 21° C in water and in air. For an oxygen consumption rate of 30 ml/min/kg$^{3/4}$ the skin temperature is about 6° C in water, 8° C in air. These comparisons illustrate the general correspondence between metabolism and skin temperature in air and water.

The correspondence shown in Fig. 7 leads to the correlation between metabolism and skin temperature shown in Fig. 8, which combines results obtained both in air and in water into a composite description of seasonal changes. It is apparent that the principal differences between results obtained during the winter and those obtained during the summer is that in the latter metabolism began to increase at a higher skin temperature and was maintained at a higher level for the same skin temperature.

Insulation of Body and Medium

From the heat production, body, skin, and ambient temperatures, the insulation index (including evaporative heat loss) may be derived (Table III). Comparisons are made at the critical temperatures at which total body insulation is theoretically maximal (10). Insulation indices of body, medium, and total (body + medium) are calculated for summer and winter seals in water and for summer seals in air and in water.

The estimations show that the insulation index of the medium is some 20 times greater for air than for water but the errors in the measurements of small temperature differences in water renders the quantitative comparisons uncertain. In spite of the large difference in the cooling effects of air and water the total insulation in air is only about double that in water because the

TABLE III

Thermal properties of seals at critical temperature

(T = temperature, °C; MR = mean standard metabolism; I = insulation index)

	In water, winter	In water, summer	In air, summer
T, medium	13	20	2°
T, skin	14.5	21.0	21.0
T, body	37.0	37.0	37.0
MR, Cal/kg$^{3/4}$/min	.909	.862	.862
I, medium $= \frac{T\,(\text{skin}-\text{medium})}{MR}$	1.2	1.2	22.1
I, body $= \frac{T\,(\text{body}-\text{skin})}{MR}$	24.8	18.6	18.6
I, total $= \frac{T\,(\text{body}-\text{medium})}{MR}$	26.4	19.8	40.7

insulation index of the body is about equal in air and water at the respective critical temperatures. Finally, the insulation index of the seals' body in water appears to have been approximately 30% greater during the winter of 1955 than during the summer of 1956. The lower insulation in summer follows from the higher critical temperature and metabolic rate below the critical level observed in the seals during the summer.

Discussion

The study of the energetics of harbor seals in air and in water during winter and summer has revealed two principal new findings. The first was that increase of metabolism by cold during the summer was closely dependent upon the degree of body-skin cooling whether the medium was air or water (Fig. 7). The metabolic rates were approximately the same at comparable skin temperatures in water or in air with 20 times the cooling effect as water. It was also shown in summer that the body insulation index was the same in air or water at the critical temperature (skin temperature about 21° C). The metabolic rates could not be correlated with temperatures of the extremities, which showed a much less regular dependence on ambient temperature.

The second new finding was that, apart from two exceptional seals, the seasonal changes paralleled in direction the seasonal insulative acclimatization seen in certain land mammals with thick fur (9), i.e. the critical temperature was higher and over-all insulation was lower during the summer, and the heat production was greater over most of the temperature range. Unlike the land mammals, the seasonal change in energetics of harbor seals cannot be associated primarily with changes in insulation of the fur but with physiological adjustments in the living tissues themselves. The greater heat production at comparable cool temperatures during the summer suggests higher skin temperatures and an altered distribution of temperatures within the blubber. Yet, no seasonal differences in skin temperatures or in temperature gradients were observed, owing, presumably, to insufficient data for seals in air and to insufficient sensitivity of thermocouple measurements for seals in water. It may be appreciated that with the existing small temperature differences between skin and water (maximum 2° C), more sensitive methods would be required for the detection of seasonal changes, which might be no more than 1/2° C.

A puzzling outcome of this study was the seasonal shift in metabolic response to cold corresponding to the same skin temperature. So far as we are aware, this adjustment has not been previously reported. It is apparent that the same skin temperature corresponded to a greater heat flow in the summer than in the winter animals, and that compensatory shifts must have occurred in the avenues of heat loss. One possibility is that the temperature of the body surface was not changed, but more heat was lost from the appendages during the summer, through some modification in operation of the heat-exchanger system characteristic of aquatic mammals (11). Another possibility is that more heat was lost by evaporative cooling during the summer, but when the seal was in water this would have to be entirely from the lungs.

The seasonal changes suggested above can be regarded as extensions of the immediate compensations to changes in ambient temperature. As the body skin temperature increased, above critical temperature, heat loss from the body surface apparently decreased while heat loss through other channels (appendages and possibly evaporation) must have increased. This can be deduced from the fact that metabolism (and total heat loss) was independent of ambient temperature at skin temperatures above about 21° C (Fig. 8), yet the temperature difference between skin and ambient (and hence heat flow) decreased as the skin temperature increased above 21° C (Fig. 2). Direct measurements of heat flow from the body surface in water also suggest decreasing flow through that surface with increasing temperature in the thermoneutral zone, but the data were insufficient for final decision.

The central importance of skin temperatures in the metabolic responses of seals raises important problems concerning the stimulation of metabolism through peripheral nervous impulses. The approximate equality of metabolism in air and water at equal skin temperatures conforms with the view (1, 2) that thermogenesis is determined by peripheral impulses that are dependent on temperature in the skin. The seasonal shift in metabolic response for the same skin temperature implies a shift in nervous impulses with greater peripheral stimulation of metabolism in summer animals. It is unfortunate that integrative methods do not exist for assessing peripheral nervous stimulation, for very large species differences must exist, particularly between well-furred species with regularly warm skin and "bare skinned" species with variably cold skin.

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References

1. Bazett, H. C. Theory of reflex controls to explain regulation of body temperature at rest and during exercise. J. Appl. Physiol. **4**, 245–262 (1951).
2. Carlson, L. D. Man in cold environment. Arctic Aeromed. Lab. Monog. 77–79 (1954).
3. Hart, J. S. Seasonal changes in insulation of the fur. Can. J. Zool. **34**, 53–57 (1956).
4. Hart, J. S. Climatic and temperature induced changes in the energetics of homeotherms. Rev. can. biol. **16**, 133–174 (1957).
5. Hart, J. S. and Heroux, O. A comparison of some seasonal and temperature changes in *Peromyscus;* cold resistance, metabolism, and pelage insulation. Can. J. Zool. **31**, 528 (1953).
6. Irving, L. Physiological insulation of swine as bare-skinned mammals. J. Appl. Physiol. **9**, 414–420 (1956).
7. Irving, L. and Hart, J. S. The metabolism and insulation of seals as bare-skinned mammals in cold water. Can. J. Zool. **35**, 497–511 (1957).
8. Irving, L. and Krog, J. Temperature of skin in the arctic as a regulator of heat. J. Appl. Physiol. **7**, 355–365 (1955).
9. Irving, L., Krog, J., and Monson, M. The metabolism of some Alaskan animals in winter and summer. Physiol. Zool. **88**, 173 (1935).
10. Scholander, P. F., Hock, R., Walters, V., Johnson, F., and Irving, L. Heat regulation in some arctic and tropical mammals and birds. Biol. Bull. **99**, 237–258 (1950).
11. Scholander, P. F. and Schevill, W. E. Countercurrent vascular heat exchange in the fins of whales. J. Appl. Physiol. **8**, 279–282 (1955).
12. Scholander, P. F., Walters, V., Hock, R., and Irving, L. Body insulation of some arctic and tropical mammals and birds. Biol. Bull. **99**, 225–236 (1950).

PHOCANEMA, A NEW GENUS FOR THE ANISAKID NEMATODE OF SEALS[1]

BETTY J. MYERS

Abstract

The anisakid nematode originally called *Ascaris decipiens* Krabbe, 1878, the adults of which occur in seals in the Atlantic off the eastern coast of Canada, is removed from the genera *Porrocaecum* and *Terranova* in which it has been variously placed and is made the type and only species of the new genus *Phocanema*. The hosts of the genus *Porrocaecum* are birds and the hosts of the genus *Terranova* are elasmobranchs.

The earliest record of an ascarid from seals was that of Krabbe (20, 21), who described *Ascaris decipiens* from six species of seals from Greenland. A new species, *Ascaris bulbosa*, from the collections of nematodes made by Kükenthal was described by Cobb (6, 7). Von Linstow (24) described *A. simplex* from *Otaria jubata* in material collected by the *Challenger* expedition, expressing surprise that *A. simplex*, a species normally found in dolphins, should be found in seals.

Jägerskiöld (14) identified specimens from *Halichoerus grypus* from the Zoological Institute of Upsala University as *A. decipiens*.

Stiles and Hassall (43), in cataloguing specimens of the Leidy Collection, identified *A. decipiens* nematodes from *Mirounga leonina* (*Macrorhinus angustirostris*), as well as from *Phoca vitulina* from the Leipzig Museum collection. In their review of the parasites of the fur seal, Stiles and Hassall (44) stated:

> "The observations by Krabbe, Cobb, and Jägerskiöld seem to be the only original work upon *A. decipiens* which has been published, and in this connection it must be recalled that the specific identity of the worms described by Krabbe and Jägerskiöld is probably but not absolutely established; furthermore that Rudolphi's (1809) original *A. simplex* might possibly belong to this species rather than to *A. angulivalis*."

Railliet and Henry (36) reported *A. decipiens* from seals of the Antarctic. A species known as *A. rectangula* was described by von Linstow (25) from material collected during the *Scotia* expedition. *Physaloptera guiarti* from material collected by the Belgian Antarctic Expedition was described by Garin (11) but was later identified as *A. decipiens* by Johnston (15).

After re-examination and redescription of the species described as *Ascaris simplex* (24) Baylis (1) considered it to be *A. decipiens*. He concluded that it would probably be found to belong to the genus *Terranova* Leiper and Atkinson, 1914, but retained the name *A. decipiens*. He later (2) suppressed the genus *Terranova* as a synonym of *Porrocaecum* and referred *A. decipiens* to it.

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Adult specimens were identified by Scheffer and Slipp (38) from *Phoca vitulina richardi* taken from Puget Sound, Washington.

In a discussion of the ascarids parasitic in seals, Baylis (4) concluded that the seal referred to as *Otario jubata* by von Linstow (24) was really *Arctocephalus gazella*. After examination of the original material described by von Linstow as *Ascaris rectangula*, he found that most of the specimens belonged to the genus *Porrocaecum* and only a few specimens were species of *Contracaecum*.

Unaware of this work Johnston (15) discussed the validity of *A. rectangula* and the misidentification of the host by von Linstow, and came to the same conclusions as Baylis. He also listed the seals in the Antarctic which were parasitized by *P. decipiens*.

Material collected from *Phocaena phocaena* was considered to be *Porrocaecum* by Schmidt-Ries (39). Lyster (26) recorded *P. decipiens* from seals of the Hudson Bay region and Herman (13) reported worms of this genus from *Zalophus* sp. of California.

Johnston and Mawson (17) concluded that *Terranova piscium* (Rud.) Johnston and Mawson, 1943 was the valid name for *P. decipiens*.

The sea otter (*Enhydra lutris nereis*) of Alaska was reported as a definitive host by Rausch (37).

The occurrence of *Terranova decipiens* in seals of the northern Pacific was reported by Margolis (27), who considered *Porrocaecum azarasi* Yamaguti and Arima, 1942 and *P. callotariae* Yamaguti, 1951 to be synonyms of *T. decipiens*.

Myers (32, 33) discussed the distribution of *P. decipiens* in the north Atlantic Ocean. Scott (41) considered all species of *Terranova* to be members of the genus *Porrocaecum*.

Due to the existing confusion in the taxonomic position of this species it seems desirable to discuss the validity of the species originally described as *Ascaris decipiens* Krabbe, 1878.

In his review of the classification of the Ascaridae Baylis (2) placed emphasis on the use of the oesophageal structures as a basis for the separation of the genera of the Ascaridae. Upon re-examination of the type species of *Terranova*, *T. antarctica* Leiper and Atkinson, 1914, he observed that the oesophagus possessed a ventricular portion and for this reason placed *Terranova* as a synonym of *Porrocaecum*. He did not consider the absence of interlabia in *Terranova* as significant and therefore considered *A. decipiens* to be a species of the genus *Porrocaecum*.

Baylis and Daubney (5), Yorke and Maplestone (46), Cram (8), and Baylis (3) suppressed *Terranova* as a synonym of *Porrocaecum* and the generic diagnosis was modified from "Interlabia present" to "Interlabia usually present."

Johnston and Mawson (18) restricted the genus *Porrocaecum* to species possessing interlabia (this confined the genus to species occurring in birds); they preferred to use the generic diagnosis of Cram (8):

> "Esophagus with anterior muscular portion and a posterior ventriculus of oblong shape, the latter short in the genotype but in other species frequently long and bent at an angle so as to open into the intestine laterally. Intestinal caecum present. Esophageal appendix absent. Interlabia present, usually small. Dentigerous ridges usually present.
>
> "Type species: *P. crassum* (Deslongchamps, 1824) Railliet and Henry, 1912."

Johnston and Mawson retained the genus *Terranova* for species lacking interlabia; these species are restricted to elasmobranch, fish, and seal hosts. They did not, however, redefine the genus.

The genus *Terranova* established by Leiper and Atkinson (22, 23) was based on a single female from the stomach of *Mustelus antarcticus* (elasmobranch) collected during the *Terra Nova* expedition to the Antarctic. Their generic diagnosis is as follows:

> "An ascarid with three simple lips. No interlabia. Oesophagus simple. Gut with an anterior caecal prolongation. No oesophageal appendix. Type species: *Terranova antarctica.*"

Karokhin (19) divided the genus *Porrocaecum* into two subgenera: the subgenus *Terranova* for those species lacking interlabia and the subgenus *Porrocaecum* for those possessing interlabia. The same subgeneric division was used independently by Dollfus (9).

Olsen (34), with the transfer of two species of *Porrocaecum* described by Chandler and the description of a new species, raised the number of species of *Terranova* to 12.

The species of *Porrocaecum* recognized by Johnston and Mawson (18) all possess interlabia and are confined to aquatic and predatory birds. The only known life cycle is that of *P. crassum* of birds, which occurs as an adult in ducks and utilizes the earthworm as an intermediate host (30). Osche (35) verified the hypothesis of Schwartz that the larvae in rodents became adults in birds of prey. Larvae of *P. decipiens* have been found in marine fish and these were shown by Schiffman (personal communication) and subsequently by Scott (40) to be infective to the seal host. Life cycle studies available present no evidence whereby *P. decipiens* can be placed in the proper genus.

At present the main separation of *Terranova* and *Porrocaecum* is based on the presence or absence of interlabia. Olsen (34) in his description of *T. ginglymostomae* from the shark *Ginglymostoma cirratum* (Gmelin) described the cephalic structures as follows:

> "Lips of type characteristic of the genus, being poorly demarked from the neck region and each bearing a median bidentate projection with dentigerous border. Dorsal lips bear two large papillae near the lateral margins; subventrals one large papilla each, on the ventral side. No interlabia present. A cuticular membrane appears to join the subventral lips ventrally."

Johnston and Mawson (16) described *Porrocaecum kogiae* from the pigmy whale (*Kogia breviceps*). It resembled *Porrocaecum decipiens* in cephalic structure but differed in being shorter in length, in the position of the vulva (a short distance behind the oesophagus), in the opening of the excretory pore at the level of the nerve ring, and in the presence of unequal spicules and a gubernaculum in the male. They subsequently (18) included it in the genus *Terranova*. Mozgovoi (28) established a new genus *Pseudoterranova* for this species from the whale.

A review of the descriptions of the species and *Terranova* in elasmobranchs and fishes has shown basic differences between those species and *P. decipiens* of seals. These differences exist in the cephalic region and in the tail region of the male. Because of these the new genus *Phocanema* is proposed with *P. decipiens* (Krabbe, 1878) as its type and only species. It differs from *Porrocaecum* in its restricted sense, by the lack of interlabia; this genus is confined to parasites of birds.

Phocanema nov. gen.

Anisakinae. Three prominent, well-developed bilobed lips present. Dentigerous ridges present. Interlabia and teeth absent. Excretory pore opens at the base of the subventral lips. Cervical alae absent. Ventriculus dilated anteriorly to form a pseudobulb, preceded by an oblong portion. Anterior projecting intestinal caecum present. Vulva opens in the anterior third of the body. Spicules subequal. Three postcloacal dentigerous ridges present.

Parasites of marine mammals.

Type and only species: *Phocanema decipiens* (Krabbe, 1878) n. comb.

Synonyms (based on adult specimens only):

Ascaris bulbosa Cobb, 1888,
Ascaris decipiens Krabbe, 1878,
Ascaris rectangula von Linstow, 1907 nec Leiper and Atkinson, 1915,
Ascaris simplex von Linstow, 1888 (host *Otaria jubata*),
Physaloptera guiarta Garin, 1913,
?*Porrocaecum azarasi* Yamaguti and Arima, 1942,
?*Porrocaecum callotariae* Yamaguti, 1951,
Porrocaecum decipiens (Krabbe, 1878) Baylis, 1920,
Terranova decipiens (Krabbe, 1878) Mozgovoi, 1953,
Terranova piscium (Rud., 1809) Johnston and Mawson, 1943.

Mozgovoi (31) incorrectly listed the species as *Terranova decipiens* (Krabbe, 1878) Baylis, 1916. Baylis retained the name *Ascaris decipiens*.

Hosts in the North Atlantic Infected with *Phocanema decipiens:*

Cystophora cristata (Erxleben) (20),
Erignathus barbatus (Erxleben) (20, 26, 33),
Halichoerus grypus (O. Fabricius) (42),
Phoca gröenlandica Erxleben (20, 32, 42),

Phoca vitulina concolor DeKay (20, 26, 33, 42),
Phoca hispida soperi Anderson (20, 33),
Odobenus rosmarus rosmarus L. (20).

Hosts in the North Pacific Infected with *Phocanema decipiens:*
Callorhinus ursinus cynocephalus (Walbaum) (44),
Enhydra lutris nereis (Merriam) (37),
Erignathus barbatus (Erxleben),
Eumetopias jubata (Schreber) (27),
Phoca vitulina richardi (Gray) (10, 27, 38, 44),
Zalophus californianus (13).

Hosts in the South Pacific Infected with *Phocanema decipiens:*
Arctocephalus gazella (Peter) (1),
Arctocephalus hookeri Gray (17),
Hydrurga leptonyx Blainville (15),
Leptonychotes weddelli Lesson (11, 15, 18, 36),
Mirounga leonina L. (15, 18).

Because nematodes of the subfamily Anisakinae occur in a variety of marine fish-eating vertebrates, search was made for species of *Phocanema* in the following animals. In no case were adult *Phocanema* found.

Elasmobranchs.—Ninety-two specimens of spiny dogfish (*Squalus acanthias* Linnaeus, 1758), 3 mackerel sharks (*Lamna nasus* Bonnaterre, 1788), 29 barndoor skates (*Raja laevis* Mitchill, 1817), 69 big skates (*Raja ocellata* Mitchill, 1815), and 90 thorny skates (*Raja radiata* Donovan, 1807) from the Magdalen Islands, Quebec, and 80 blue sharks (*Prionace glauca* Linnaeus, 1758) from Nova Scotia.

Birds.—Twenty-eight specimens of cormorants (*Phalacrocorax auritus*), four seagulls (*Larus* sp.), three gannets (*Sula bassana*), one loon (*Gavia* sp.), and one eider duck (*Somateria mollissima*) from the Magdalen Islands, Quebec. Scott (41) found no *Porrocaecum* in the stomachs of cormorants from the Miramichi estuary, New Brunswick. He also reported that *Porrocaecum* was not found in: "eleven species of cormorants, one of penguins, two of loons, four of grebes, four of turbinares, three of mergansers, nine of gulls and terns and three of auks...." The only record of any species of *Porrocaecum* from cormorants is Yamaguti's (45) description of *P. phalacrocoracis* from *Phalacrocorax capillatus* of Japan. Heller (12) found no *Porrocaecum* larvae or adults in 20 *Raja radiata*, 1 *R. laevis*, and 3 *Squalus acanthias* from the Baie of Chaleur, Quebec. Previous workers had similar negative findings.

Porpoises.—Scott and Fisher (42) found in an examination of 150 porpoises that 25% of the larvae collected were *Porrocaecum* and doubtfully reported one *P. decipiens* male from the stomach. Eighty larval *Porrocaecum* but no adults were reported from the stomach of the white whale (*Delphinapterus leucas*); Lyster (26) reported no adult or larval *Porrocaecum* from white whales collected in the Gulf of St. Lawrence.

Experimental infections.—Experimental infections of feeding *Phocanema*-type larva from cod to cormorants and skates gave negative results. These results agree with the suggestion that the genus *Phocanema* is restricted as an adult to seals in the western north Atlantic ocean.

References

1. BAYLIS, H. A. Some ascarids in the British Museum (Natural History). Parasitology, **8**, 360–378 (1916).
2. BAYLIS, H. A. On the classification of the Ascaridae. I. The systematic value of certain characters of the alimentary canal. Parasitology, **12**, 253–264 (1920).
3. BAYLIS, H. A. Fauna of British India including Ceylon and Burma. I. Ascaroidea and Strongyloidea. Secretary of State for India in Council, London. 1936.
4. BAYLIS, H. A. On the ascarids parasitic in seals with special reference to the genus *Contracaecum*. Parasitology, **29**, 121–130 (1937).
5. BAYLIS, H. A. and DAUBNEY, R. A synopsis of the families and genera of Nematoda. Trustees of the British Museum, London. 1926.
6. COBB, N. A. Beiträge zur Anatomie und Ontogenie der Nematoden. Jena. Z. Naturw. **23**, n.s. **16**, 41–76 (1888).
7. COBB, N. A. Neueparasitische Nematoden (*In* Kükenthal. "Beiträge zur Fauna Spitzbergens"). Arch. Naturgeschichte, Berlin, 55, J. **1**, 149–159 (1889).
8. CRAM, E. B. Bird parasites of the nematode suborders Strongylata, Ascaridata and Spirurata. U.S. Natl. Museum Bull. 140 (1927).
9. DOLLFUS, R. P. Aperçu général sur l'histoire naturelle des parasites animaux de la morue Atlanto-Arctique *Gadus callarias* L. (= *Morhua* L.) et leur distribution géographique. (Encyclopédie biologique No. 43.) Paul Lechevalier, Paris. 1953.
10. FISHER, H. D. The status of the harbour seal in British Columbia with particular reference to the Skeena River. Bull. Fisheries Research Board Can. No. 93 (1952).
11. GARIN, C. P. Récherches physiologiques sur la fixation et le mode de nutrition de quelques nématodes parasites du tube digestif de l'homme et des animaux. Ann. Univ. Lyon, n.s. 1, Sc. Méd. Fasc. **34**, 1–160 (1913).
12. HELLER, A. F. Parasites of cod and other marine fish from the Baie of Chaleur region. Can. J. Research, D, **27**, 243–264 (1949).
13. HERMAN, C. M. The effect of higueronia on nematodes and nemathelmintic gastric ulcers of California sea lions. Rev. med. trop. parasitol., Bacteriol. Clin. Lab. Habana, **8**, 45–47 (1942).
14. JÄGERSKIÖLD, L. A. Beiträge zur Kenntnis der Nematoden. Zool. Jahrb. Jena. Abt. Anat. **7**, 449–532 (1894).
15. JOHNSTON, T. H. Parasitic nematoda. Australasian Antarctic Exped. (1911–14). Sci. Rept. Ser. C, Zool. and Bot. (1937), **10**, 1–31 (1938).
16. JOHNSTON, T. H. and MAWSON, P. M. Internal parasites of the pigmy sperm whale. Record South Australian Museum, **6**, 263–274 (1939).
17. JOHNSTON, T. H. and MAWSON, P. M. Endoparasites from the subarctic islands of New Zealand. Record South Australian Museum, **7**, 237–243 (1943).
18. JOHNSTON, T. H. and MAWSON, P. M. Parasitic nematodes. Rept. B.A.N.Z. Antarctic Research Expedition, 1929–1931, Ser. B, **5**, 73–159 (1945).
19. KAROKHIN, V. I. Two new species of *Porrocaecum* from birds of prey in Siberia. Gel'mint. Sborn. 40-Let. Deiatel'nost. Skrjabin, 135–141 (1946). (In Russian.)
20. KRABBE, H. Saelernes og Tandhvalernes Spolorme. Overs. Kl. Danske Videnskab. Selskab. Forh. **1**, 43–51 (Résumé in French 11–12) (1878).
21. KRABBE, H. On ascarids of the seals and toothed whales. Ann. Mag. Nat. Hist. 5 s. **2**, 430–432 (1878). (Abstract of reference No. 20.)
22. LEIPER, R. T. and ATKINSON, E. L. Helminthes of the British Antarctic Expedition 1910–1913. Proc. Zool. Soc. London, **1**, 222–226 (1914).
23. LEIPER, R. T. and ATKINSON, E. L. Parasitic worms with a note on a free living nematode. Brit. Museum (Nat. Hist.) British Antarctic (Terra Nova) Expedition 1910. Nat. Hist. Rept. Zool. **2**, 19–60 (1915).
24. VON LINSTOW, O. Report on Entozoa collected by H. M. S. Challenger during the years 1873–76. Rept. Voyage H. M. S. Challenger (1873–76), **23** (71), 1–18 (1888).
25. VON LINSTOW, O. Nematodes of the Scottish National Antarctic Expedition 1902–1904. Proc. Roy. Soc. Edinburgh (1905–06), **26**, 464–472 (1907).
26. LYSTER, L. L. Parasites of some Canadian sea mammals. Can. J. Research, D, **18**, 395–409 (1940).
27. MARGOLIS, L. Parasitic helminths and arthropods from Pinnipedia of the Canadian Pacific coast. J. Fisheries Research Board Can. **13**, 489–505 (1956).

28. Mozgovoi, A. A. Ascaridata of animals. Morphology, biology, systematics and an attempt to construct their phylogenetics of zoogeography. (Abstract.) Trudy Gel'mint. Lab. Akad. Nauk. S.S.S.R. **4**, 263–269 (1950). (In Russian.)
29. Mozgovoi, A. A. Ascaridata (Anisakoidea) of mammals in the U.S.S.R. Trudy Gel'mint Lab. Akad. S.S.S.R. **5**, 14–22 (1951). (In Russian.)
30. Mozgovoi, A. A. The biology of *Porrocaecum crassum* nematodes of wild aquatic birds. Trudy Gel'mint. Lab. Akad. Nauk. S.S.S.R. **6**, 114–125 (1952).
31. Mozgovoi, A. A. Principles of nematodology. II. Ascaridata of animals and man and illnesses caused by them. Part I. (*Edited by* K. I. Skrjabin.) Izdatelstvo Akad. Nauk. S.S.S.R. 1953. (In Russian.)
32. Myers, B. J. Ascaroid parasites of harp seals (*Phoca gröenlandica* Erxleben) from the Magdalen Islands, Quebec. Can. J. Zool. **35**, 291–292 (1957).
33. Myers, B. J. Nematode parasites of seals in the eastern Canadian Arctic. Can. J. Zool. **35**, 291 (1957).
34. Olsen, L. S. Some nematodes parasitic in marine fishes. Publs. Inst. Marine Sci. **2**, 173–215 (1952).
35. Osche, G. Ueber Entwicklung Zwischenwirt und Bau von Bau von *Porrocaecum talpae*, *Porrocaecum ensicaudatum* und *Habronema mansoni* (Nematoda). Z. Parasitenk. Berlin, **17**, 144–164. 1955.
36. Railliet, A. and Henry, A. Némathelminthes parasites. (Expédition antarctique française (1903–1905) commandée par Jean Charcot). Paris. 1907.
37. Rausch, R. L. Studies of the helminth fauna of Alaska. XIII. Diseases in the sea otter, with special reference to helminth parasites. Ecology, **34**, 584–604 (1953).
38. Scheffer, T. H. and Slipp, J. W. The harbor seal in Washington State. Am. Midland Naturalist, **32**, 373–416 (1944).
39. Schmidt-Ries, H. Die bisher bei dem Kleinen Tümmler (*Phocaena phocaena* L.) festgestellten Parasiten. Zentr. Bakteriol. I. Abt. Orig. **145**, 89–106 (1939).
40. Scott, D. M. Experiments with the harbour seal, *Phoca vitulina*, a definitive host of a marine nematode, *Porrocaecum decipiens*. J. Fisheries Research Board Can. **10**, 539–547 (1953).
41. Scott, D. M. On the specific identity of the larval *Porrocaecum* (Nematoda) in Atlantic cod. J. Fisheries Research Board Can. **13**, 343–356 (1956).
42. Scott, D. M. and Fisher, H. D. Incidence of a parasitic ascarid *Porrocaecum decipiens* in the common porpoise *Phocoena phocoena* from the lower Bay of Fundy. J. Fisheries Research Board Can. **15** (1), 1–4 (1958).
43. Stiles, C. W. and Hassall, A. A preliminary catalogue of the parasites contained in the collections of the United States Bureau of Animal Industry, United States Army Medical Museum, Biological Department of the University of Pennsylvania (collection Leidy), and in collection Stiles and Hassall. Vet. Mag. **1**, 245–253, 331–354 (1894).
44. Stiles, C. W. and Hassall, A. Internal parasites of the fur seal. (*In* The fur seals and fur seal islands of the North Pacific Ocean, by David S. Jordan, *et al.*). Part 3. Washington. 1899. pp. 99–177.
45. Yamaguti, S. Studies on the helminth fauna of Japan. Part 36. Avian nematodes II. Japanese J. Zool. **9** (3), 441–480 (1941).
46. Yorke, W. and Maplestone, P. A. The nematode parasites of vertebrates. P. Blakiston's Son & Co., Philadelphia. 1926.

PROTOZOA, INCLUDING AN ENTOZOAN OF GUINEA PIGS, FROM HOUSE FLIES[1]

MARSHALL LAIRD

Abstract

Of 179 examples of *Musca domestica* L. examined, 7 harbored *Octosporea muscae-domesticae* Flu (Sporozoa : Microsporidia), 2 *Herpetomonas muscarum* (Leidy) (Mastigophora : Protomonadina), and 2 *Retortamonas caviae* (Hegner and Schumaker) (Mastigophora : Retortamonadina). Trophozoites of the last-named species, a common entozoan of guinea pigs, were active in the mid-gut and faeces of the flies concerned, which had presumably ingested the trophozoites from moist, fresh faeces of the normal host.

Between February and May, 1958, 179 house flies (*Musca domestica* L.) were collected in the animal quarters of the Institute of Parasitology and examined for Protozoa. Seven of them proved to be parasitized by the microsporidian *Octosporea muscae-domesticae* Flu and two harbored the trypanosomatid flagellate *Herpetomonas muscarum* (Leidy).

The first of these organisms, already recorded from the same host in the Montreal area (3), was found in the fat body and malpighian tubules. Rosettes of eight binucleate sporoblasts, of a diameter of from 8.5 to 11 μ when fresh, were not uncommon. The dimensions of the oval to arcuate spores (av., 6 μ by 1 μ in the fresh state) agree with figures published by Chatton and Krempf (1), but the breadth is relatively less than that of the examples seen in Quebec by Fantham and Porter (2 to 3 μ).

Herpetomonads were common in the mid-gut of the two flies concerned. Fifty consecutive examples in a Giemsa-stained dry film measured from 16.9 to 28.8 μ (av., 21.5 μ) by from 2.1 to 4.3 μ (av., 3.3 μ). Longitudinal cytoplasmic cleavage had commenced in some of the widest ones, and many others (about 40% of the total) had two flagella. Omitting predivision forms from consideration, a lesser average width figure (3.0 μ) was obtained. The length of the free flagellum ranged from one and one-half to three times that of the body proper, the longest for the 50 examples concerned measuring 61 μ. These figures and the general morphology of the organism are in accord with descriptions of *Herpetomonas muscarum* by several authors. A strain of appreciably smaller size, 12 to 18.5 μ (av., 16 μ) by 2 μ, was recently described from *Phaenicia sericata* (Meigen) in the U.S.A. (8).

Of rather more interest than the discovery of these well-known and cosmopolitan parasites was that of a few active trophozoites of a species of *Retortamonas* in the mid-gut and faeces of two flies collected on February 16 and May 21. Eleven haematoxylin-stained examples measured from 4.5 to 7.2 μ by from 3.9 to 4.2 μ. Biflagellates of the genus—formerly placed in the Protomonadina, but lately transferred to Grasse's new order, Retortamonadina (4)—inhabit the intestine of certain insects and vertebrates.

[1]Manuscript received May 6, 1959.
Contribution from the Institute of Parasitology, Macdonald College P.O., Que., with financial assistance from the National Research Council of Canada.

Muscoid flies are not numbered among their definitive hosts, although trophozoites of another member of the order, the human entozoan *Chilomastix mesnili* (Wenyon), may pass unharmed through the alimentary tract of *M. domestica* (7).

Several rabbits and a large number of guinea pigs were being maintained in the animal house at the time. Both of these laboratory animals commonly harbor *Retortamonas*. *R. cuniculi* (Collier and Boeck) of the former host is a relatively large species the trophozoites of which measure 7.5 to 13.0 μ by 5.5 to 9.5 μ (2). The dimensions of stained trophozoites of *R. caviae* (Hegner and Schumaker), however, have been given as 4 to 7 μ by 2 to 4 μ (5) and 4.4 to 7.7 μ by 4.0 to 4.3 μ (6). These figures agreeing so closely with mine, it is held that the retortamonad from *M. domestica* is in fact referable to *R. caviae*. The flies presumably ingested the trophozoites from moist, fresh faeces. No cysts were present in either case, and retortamonads must be encysted to be capable of infecting their mammalian hosts. The present record, while being the first for this particular association, thus adds nothing to our knowledge of the role of flies as agents in the spread of parasites of other animals.

References

1. CHATTON, E. and KREMPF, A. Sur le cycle évolutif et la position systématique des protistes du genre Octosporea Flu, parasites des muscides. Bull. soc. zool. France, **36**, 172–179 (1911).
2. COLLIER, J. and BOECK, W. C. The morphology and cultivation of *Embadomonas cuniculi* n. sp. J. Parasitol. **12**, 131–140 (1926).
3. FANTHAM, H. B. and PORTER, A. Some pathogenic bacteriform microsporidia from Crustacea and Insects. Proc. zool. soc. London, **130**, 153–168 (1958).
4. GRASSE, P.-P. Phylogénie. Protozoaires: Généralités. Flagellés. Traité de Zoologie Vol. I, Fasc. I. Masson et Cie, Paris. 1952.
5. HEGNER, R. and SCHUMAKER, E. Some intestinal amoebae and flagellates from the chimpanzee, three-toed sloth, sheep and guinea-pig. J. Parasitol. **15**, 31–37 (1928).
6. NIE, D. Morphology and taxonomy of the intestinal Protozoa of the guinea-pig, *Cavia porcella*. J. Morphol. **86**, 381–493 (1950).
7. ROOT, F. M. Experiments on the carriage of intestinal Protozoa of man by flies. Am. J. Hyg. **1**, 131–153 (1921).
8. WALLACE, F. G. and CLARK, T. B. Flagellate parasites of the fly, *Phaenicia sericata* (Meigen). J. Protozool. **6**, 58–61 (1959).

NEMATODE PARASITES OF VERTEBRATES OF EAST PAKISTAN

I. OXYURIDAE FROM LIZARDS (GEKKO AND HEMIDACTYLUS)[1]

S. P. GUPTA[2]

Abstract

Pharyngodon kuntzi sp. nov. from *Gekko gecko*, *P. frenatusi* sp. nov. from *Hemidactylus frenatus*, and *Thelandros* sp. from *G. gecko* are described.

The parasites described in this and subsequent papers of the series were forwarded to Dr. T. W. M. Cameron as Consultant in Parasitology, U.S. Naval Medical Research Unit No. 2, Taipei, Taiwan, and handed to me for study. I am indebted to Dr. G. A. Schad, of the Institute of Parasitology, for his invaluable help in assisting me to locate references and in the preparation of the manuscripts in this series.

To my knowledge the genus *Pharyngodon* Diesing, 1867 has not previously been recorded from Pakistan.

Thirty-eight species comprise this genus. Of the seven described from amphibians the male is known in only one—*Pharyngodon bassi* (Walton, 1940). Thirty-one species have been described from lizards and snakes. *P. bassi* differs from other species of the genus in having four instead of three pairs of cloacal papillae and it may prove to belong to a separate genus (12).

Calvente (2) subdivides the genus *Pharyngodon* into two subgenera, *Pharyngodon* (*Pharyngodon*), in which the spicule is clearly visible, and *Pharyngodon* (*Neyrapharyngodon*) in which the spicule is invisible or absent. He lists, in the form of a tabular chart, the species belonging to the two subgenera. Read and Amrein (8) are against dividing the genus on the basis of the presence or absence of the spicule. I agree with these authors because of the difficulty of placing oxyurids with weakly chitinized spicules. Read and Amrein (8) have, without comment, transferred *P. medinae* Calvente, 1948 to the genus *Parathelendros*. I disagree with this in that *P. medinae* has the characters of the genus *Pharyngodon*. I also disagree with Read and Amrein, who consider *P. australis* Johnston and Mawson, 1942 to be a synonym of *P. tiliquae* Baylis, 1930 solely on the basis of being described from the same host. *P. australis* (6) differs from *P. tiliquae* in that the caudal alae of the male of the latter include postanal papillae while the former do not. *P. tiliquae* also differs from *P. australis* in the size of the body, the position of the vulva, and the size of the eggs.

[1]Manuscript received April 6, 1959.
Contribution from the Institute of Parasitology, McGill University, Macdonald College P.O., Que., Canada. The parasites upon which this paper is based were collected by Dr. Robert E. Kuntz (Cdr. MSC, USN; U.S. Naval Medical Research Unit No. 2, Taipei, Taiwan) a member of the U.S. Naval Medical Mission to East Pakistan in 1958. He was assisted by James Reese, HM, IC, USN, and Charles Knight, HCM, USN, also of NAMRU-2. Dr. Robert F. Inger, Curator of Reptiles, Chicago Natural History Museum, has provided the identifications of the reptiles.
[2]National Research Council of Canada Postdoctorate Fellow.

Chabaud and Golvan (3) give a useful key to the species of the genus *Pharyngodon*. They consider the most primitive forms are those in which the caudal alae and caudal papillae are well developed and the most evolved forms, those in which these characters have more or less completely atrophied. They consider the species of *Pharyngodon* of primitive lizards (gekkos) and those of evolved lizards (skinks) to have quite different geographical distributions. All the Australian species belong to the primitive group as does one species in Asia. On the other hand, the Ethiopian species are members of the most evolved group. All other species in America and in the Mediterranean region are mixed and in transition between the two extremes.

Pharyngodon kuntzi sp. nov.

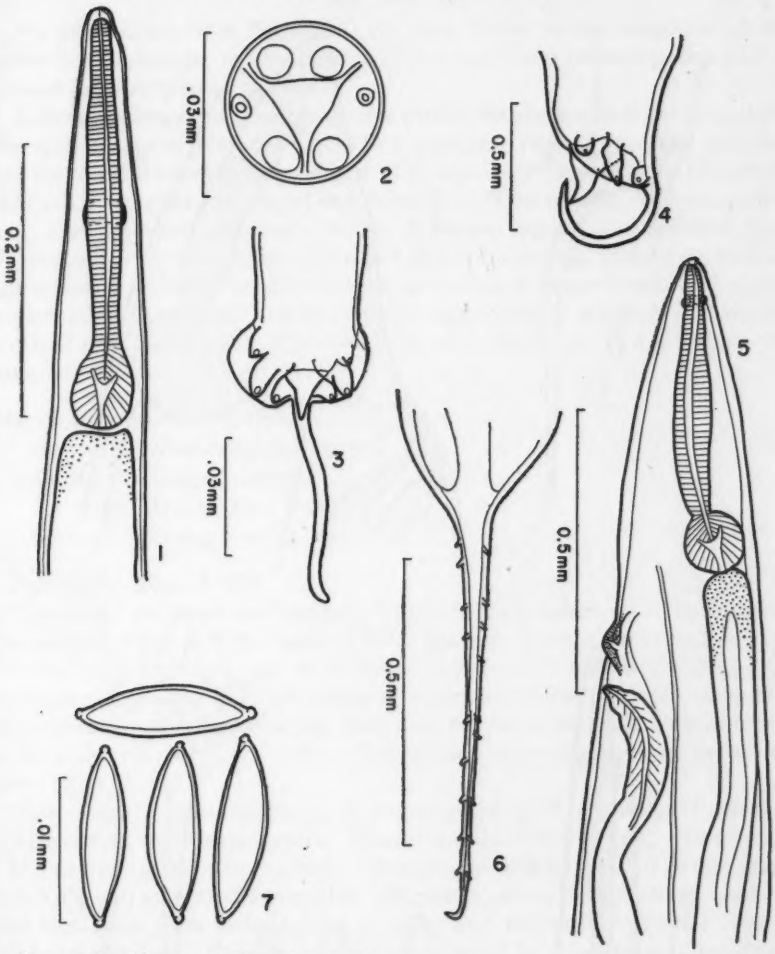
Host: *Gekko gecko.*
Location: Large intestine.
Locality: Dacca, East Pakistan.
Material: 60 females, 12 males.

Description (Figs. 1–7)

The worms are small and thin. The body tapers towards each extremity. The cuticle has fine transverse striations. The mouth is bounded by three distinct lips; there is no buccal cavity. The oesophagus is club-shaped (Fig. 1) with a posterior bulb containing a valvular apparatus and separated from the rest by a slight constriction. In both sexes the lateral alae are narrow, extending from a little posterior to the nerve ring and terminating a little anterior to the posterior end of the body. The lateral alae are not visible in the more mature females. In both sexes, there is a thin, elongated tail, that of the female having conspicuous spines (Figs. 3, 6).

Male.—1.28 to 2.25 mm long, 0.08 to 0.09 mm wide. Cuticular striations about 0.06 to 0.08 mm apart. Head (Fig. 2) 0.021 to 0.029 mm in diameter. Oesophagus, including bulb, 0.22 to 0.4 mm long. Bulb 0.04 to 0.05 mm long, 0.05 to 0.055 mm wide. Nerve ring 0.12 to 0.14 mm from anterior end of body. Excretory pore, postoesophageal, situated 0.36 to 0.59 mm from anterior end of body. Tail 0.065 to 0.08 mm long. Three pairs of caudal papillae (Figs. 3, 4) present. Postanal papillae included in caudal alae. Preanal pair sessile. Postanal pairs pedunculated. Well-developed genital cone present, about 0.13 mm long.

Female.—3.18 to 5.16 mm long, 0.38 to 0.42 mm in maximum width. Cuticular striations 0.03 to 0.11 mm apart. Diameter of head 0.035 mm to 0.05 mm. Nerve ring lies 0.05 to 0.06 mm from anterior end. Oesophagus, including bulb, 0.38 to 0.56 mm long. Bulb 0.09 to 0.1 mm long, 0.08 to 0.1 mm wide. Excretory pore (Fig. 5) situated behind oesophagus 0.58 to 0.68 mm. Vulva posterior to excretory pore, 0.7 to 0.76 mm from anterior end of body. Filamentous tail, 0.88 to 0.96 mm long, bearing 17–22 posteriorly directed cuticular spines. Eggs (Fig. 7), long, oval, with button-like knob at each pole. They measure 0.12 to 0.125 mm by 0.03 to 0.35 mm.

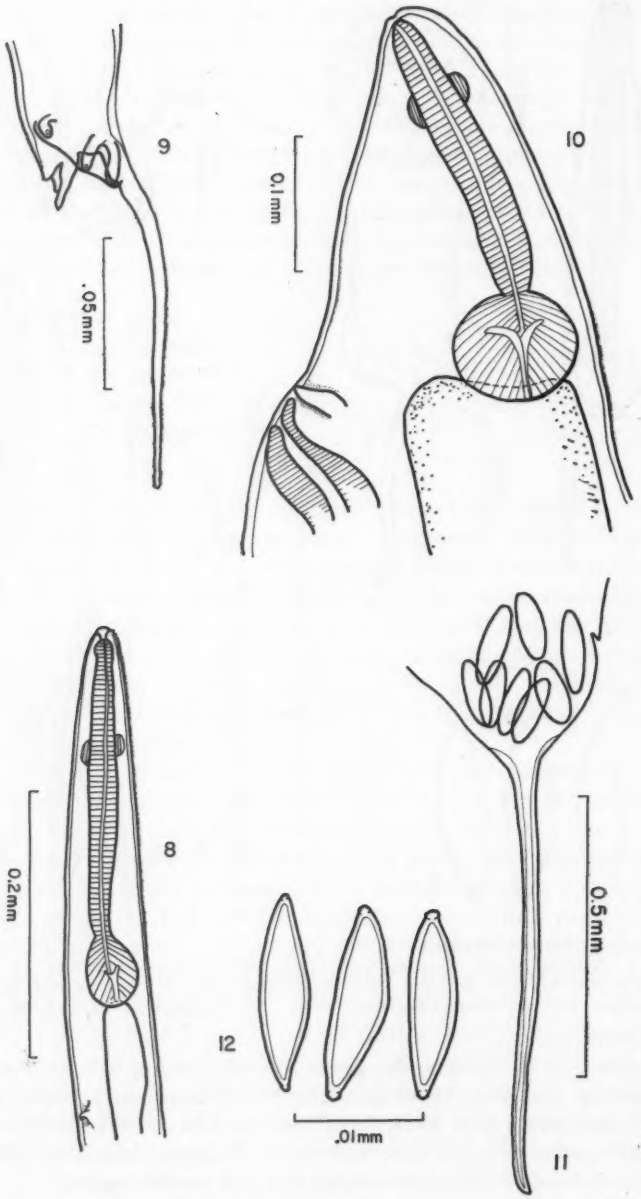


FIGS. 1–7. *Pharyngodon kuntzi* sp. nov.
FIG. 1 Anterior region of male, lateral view. FIG. 2. End-on view. FIG. 3. Male tail, ventral view. FIG. 4. Male tail, lateral view. FIG. 5. Anterior end of female, lateral view. FIG. 6. Female tail, lateral view. FIG. 7. Eggs.

Discussion

The worms are referred to the genus *Pharyngodon*, of which three species, *P. megalocerca* (Skrjabin, 1916) Seurat, 1917 (=*Oxyuris megalocerca* Skrjabin, 1916), *P. apapillosus* Koo, 1938, and *P. geckinis* Liu and Wu, 1941, are known from *Gekko gecko*. Walton (12) transferred *P. apapillosus* Koo, 1938 to the genus *Parathelandros* as it lacks caudal alae and caudal papillae.

Pharyngodon kuntzi sp. nov. differs from *P. megalocerca*, an African species, in the shape of the body, which in *P. megalocerca* consists of two distinct parts (a spindle-shaped body and an extremely thin tail (9)) in having narrow lateral alae, and in the presence of caudal alae.



FIGS. 8–12. *Pharyngodon frenatusi* sp. nov.
FIG. 8. Anterior region of male, lateral view. FIG. 9. Posterior end of male, lateral view. FIG. 10. Anterior region of female, lateral view. FIG. 11. Female tail, lateral view. FIG. 12. Eggs.

P. kuntzi differs from *P. geckinis* (7) from China, in the possession of tail spines by the female, in the absence of a spicule, and in having one pair of preanal papillae instead of two.

P. kuntzi belongs to a group of species within the genus which lacks a spicule; the caudal alae of the male include a posterior pair of postanal papillae, and the tail of the female bears spines. Two species, *P. yucatanensis* Chitwood, 1939 and *P. californiensis* Read and Amrein, 1953, possessing these characters have been reported previously (4, 8). *P. kuntzi* can be distinguished from *P. yucatanensis* by the absence of lateral alae in the female, and differs from *P. californiensis* in being smaller in size, in having a larger number of spines on the tail of the female, and in that the caudal alae of the male include the postanal papillae. In *P. californiensis* the alae reach but do not include the postanal papillae.

Pharyngodon frenatusi sp. nov.

Host: *Hemidactylus frenatus.*
Location: Large intestine.
Locality: Dacca, East Pakistan.
Materials: Seven females, one male.

Description (Figs. 8–12)

The worms are small and the body tapers to each extremity in both sexes. The mouth is bounded by three distinct lips and there is no buccal cavity. The cuticle is delicate, and transversely striated. There is a cylindrical, muscular oesophagus (Fig. 8) ending in a posterior bulb. In both sexes the lateral alae are narrow, extending from near the nerve ring to a little anterior to the posterior end of the body. Tail of both sexes filamentous, devoid of spines (Figs. 9, 11).

Male.—Smaller than female, 1.14 mm long and 0.06 mm wide. Cuticular striations 0.01 to 0.02 mm apart. Diameter of head 0.025 mm. Nerve ring 0.11 mm from anterior end of body. Oesophagus including bulb 0.28 mm long. Bulb 0.06 mm long, 0.035 mm wide. Excretory pore situated behind oesophagus, 0.37 mm from anterior end of body and bearing a cuticular fringe. Tail 0.12 mm long. Cloacal aperture surrounded by three pairs of papillae, one pair preanal and sessile, two pairs postanal and pedunculated. Postanal pairs much larger than preanal and included in caudal alae. Well-developed genital cone, about 0.18 mm long.

Female.—3.66 to 4.1 mm long, 0.4 to 0.48 mm in maximum width. Cuticular striations about 0.014 to 0.016 mm apart. Diameter of head 0.05 to 0.07 mm. Nerve ring 0.04 to 0.06 mm from anterior end. Oesophagus including bulb 0.29 to 0.3 mm long. Bulb 0.08 to 0.09 mm in diameter. Position of excretory pore (Fig. 10) from a little anterior to oesophageal bulb to level with it, 0.20 to 0.28 mm from anterior end of body. Vulva and excretory pore continguous, 0.21 to 0.29 mm from anterior end of body. Tail 0.096 to 0.1 mm long. Eggs (Fig. 12) large with knob at one pole, 0.14 to 0.155 mm long by 0.035 to 0.04 mm wide.

Discussion

Pharyngodon frenatusi sp. nov. belongs to the group of species in which the spicule is absent, anal papillae are included in the caudal alae, the excretory pore and vulva are contiguous, and the tail non-spiny. This group includes the species *P. tiliquae* Baylis, 1930 from *Tiliqua scincoides* of Queensland and *P. warneri* Harwood, 1932 from *Cnemidophorus sexlineatus* of Texas. In *P. frenatusi*, however, the postanal papillae are undivided whereas the anterior pair is forked in *P. tiliquae* (1). *P. frenatusi* also differs from *P. tiliquae* and *P. warneri* (5) in the position of the excretory pore and vulva. In *P. frenatusi* the vulva and excretory pore lie from a little anterior to the level of the oesophageal bulb to the posterior margin of it, while in *P. tiliquae* and *P. warneri* they lie behind the level of the oesophagus. *P. frenatusi* also differs from *P. warneri* in the shape of the female tail. In the latter species it tapers gradually to a sharp point whereas in the former the tail tapers abruptly and extends in a long slender filament.

Thelandros sp.

Host: *Gekko gecko.*
Location: Small intestine.
Locality: Dacca, East Pakistan.
Material: One female.

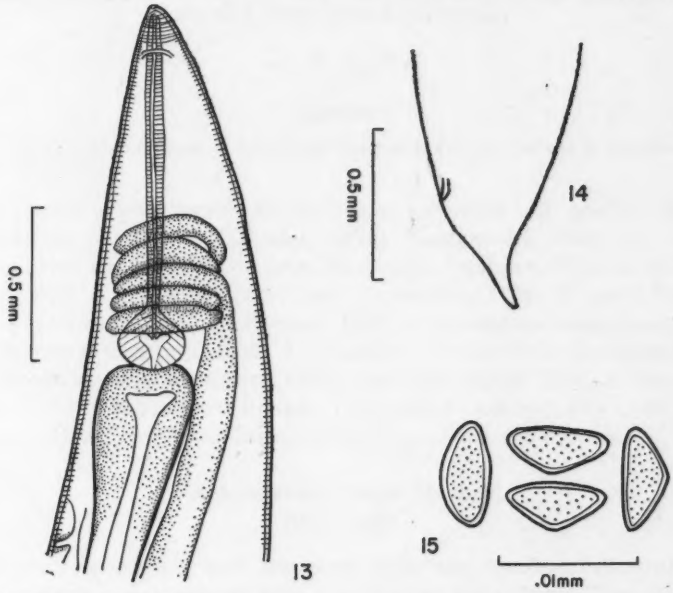
Description (Figs. 13–15)

The one worm, a female, is small, thin, and tapers towards each extremity. It measures 6.12 mm in length, 0.78 mm in maximum width. At the posterior end it possesses a short smooth tail, 0.42 mm long. Cuticular striations 0.018 to 0.02 mm apart. Excretory pore far behind oesophageal bulb, 1.8 mm from anterior end of body. Lateral alae extend from anterior part of body, and not visible behind middle region of the body due to the presence of eggs. Oesophagus (Fig. 13) long, terminating in a large, spherical bulb, 1.18 mm long to the bulb which is 0.18 mm long, 0.24 mm wide. Intestine swollen at anterior end to form a pear-shaped dilation. Nerve ring surrounds oesophagus at 0.14 mm from anterior end of body. Vulva situated in middle region of body, 2.72 mm from tip of tail, and lies far from excretory pore. Eggs (Fig. 15) convex at one side, flat on the other, 0.05 to 0.07 mm in length and 0.029 to 0.035 mm in breadth.

Discussion

This species falls into the group within the genus *Thelandros* in which the uterine tubules make definite coils around the corpus of the oesophagus (11). It closely resembles *Thelandros* sp. Malan, 1935 (=*Oxyuris* sp. Thapar, 1925) from *Tachysaurus rugosus* (10), in the size of the body, in the position of the excretory pore, and in the shape of the eggs, which are convex on one side and flat on the other. It differs in the position of the vulva, which is anterior to mid-body in *Thelandros* sp. Thapar, 1925, and in the size of the tail, which is smaller in my specimen.

As only one female is available it is impossible to assign it to a definite species.



FIGS. 13–15. *Thelandros* sp.
FIG. 13. Anterior end of female, lateral view. FIG. 14. Female tail, lateral view.
FIG. 15. Eggs.

References

1. BAYLIS, H. A. Some Heterakidae and Oxyuridae (Nematoda) from Queensland. Ann. Mag. Nat. Hist., Ser. 10, **5**, 354–366 (1930).
2. CALVENTE, I. G. Revision el genero *Pharyngodon* y descripcion de especies neuvas. Rev. ibérica parasitol. **8**, 367–410 (1948).
3. CHABAUD, A. G. and GOLVAN, Y. J. Miscellanea Helminthologica XXIV—Nématodes parasites de lézards de la forêt de Nefifik. Arch inst. Pasteur Maroc, **5**, 447–469 (1957).
4. CHITWOOD, B. G. Some nematodes from the caves of Yucatan. Publ. Carnegie Inst. Wash. **491**, 51–66 (1938).
5. HARWOOD, P. D. The helminths parasitic in the Amphibia and Reptilia of Houston, Texas and vicinity. Proc. U.S. Natl. Museum, **81**, 1–71 (1932).
6. JOHNSTON, T. H. and MAWSON, P. M. The Gallard collection of parasitic nematodes in the Australian Museum. Records Australian Museum, **21**, 110–115 (1942).
7. LIU, C. K. and WU, H. W. Notes on some parasitic nematodes. Sinensia, **12**, 61 (1941).
8. READ, C. P. and AMREIN, Y. U. North American nematodes of the genus *Pharyngodon* Diesing (Oxyuridae). J. Parasitol. **39**, 365–370 (1953).
9. SKRJABIN, K. S. Parasitic trematodes and nematodes collected by the expedition of Prof. V. Dogiel & I. Sokolov in British East Africa. Scientific Results Zool. Exp. Brit. East Africa & Uganda (V. Dogiel & I. Sokolov in 1914.) (English translation.) **1**, 99–157 (1916).
10. THAPAR, G. S. Studies on the Oxyuroid parasites of reptiles. J. Helminthol. **3**, 83–150 (1925).
11. WALTON, A. C. Distribution of the genus *Thelandros* (Nematoda: Oxyuroidea). Proc. Helminthol. Soc. Wash. **8**, 15–18 (1941).
12. WALTON, A. C. Some Oxyuroids from a Galapagos tortoise. Proc. Helminthol. Soc. **9**, 1–10 (1942).

NEMATODE PARASITES OF VERTEBRATES OF EAST PAKISTAN

II. AMPLICAECUM RANAE SP. NOV. (HETEROCHEILIIDAE RAILLIET AND HENRY, 1915) FROM AMPHIBIA[1]

S. P. GUPTA[2]

Abstract

Amplicaecum ranae sp. nov. from the intestine of *Rana tigrina* and *R. cancrivora* is described.

The genus *Amplicaecum* Baylis, 1920 comprises 15 species, namely: *Amplicaecum involutum* (Gedoelst, 1916) Sandground, 1933; *A. colorum* (Baylis, 1919) Baylis, 1920; *A. varani* Baylis and Daubney, 1922; *A. africanum* Taylor, 1924; *A. gedoelsti* Yorke and Maplestone, 1926; *A. causi* Thwaite, 1926; *A. excavatum* Hsu and Hoeppli, 1931; *A. novempapillatum* Sandground, 1933; *A. cacopi* Chatterji, 1936; *A. schoutdeni* Baylis, 1940; *A. alatum* Baylis, 1947; *A. schikhobalovi* Mozgovoi, 1950; *A. monitor* Khera, 1954; *A. numidicum* (Seurat, 1917) Chabaud and Rouget, 1955; and *A. ranae* sp. nov. All except *A. colorum* (from an African eagle) are from amphibia and reptiles.

Amplicaecum ranae sp. nov.

(Figs. 1–6)

Two male specimens were recovered from the small intestine of *Rana tigrina* and one male specimen from *R. cancrivora* from Dacca, East Pakistan.

Worms are large and stout. Cuticle thick, smooth, with fine transverse striations. Length 18.8 to 20.96 mm, width 0.3 to 0.35 mm. Head diameter 0.125 to 0.13 mm. Mouth surrounded by three well-developed rectangular lips, one dorsal, two subventral, all of which have dentigerous ridges composed of small teeth on the inner margins, which are characteristic of the genus. Interlabia absent; grooves present on outer surface of base of each lip. Dorsal lip bears two large papillae. Pulp provided internally with two anterior processes. Each ventrolateral lip with one large papilla towards ventral side and one very small papilla laterally.

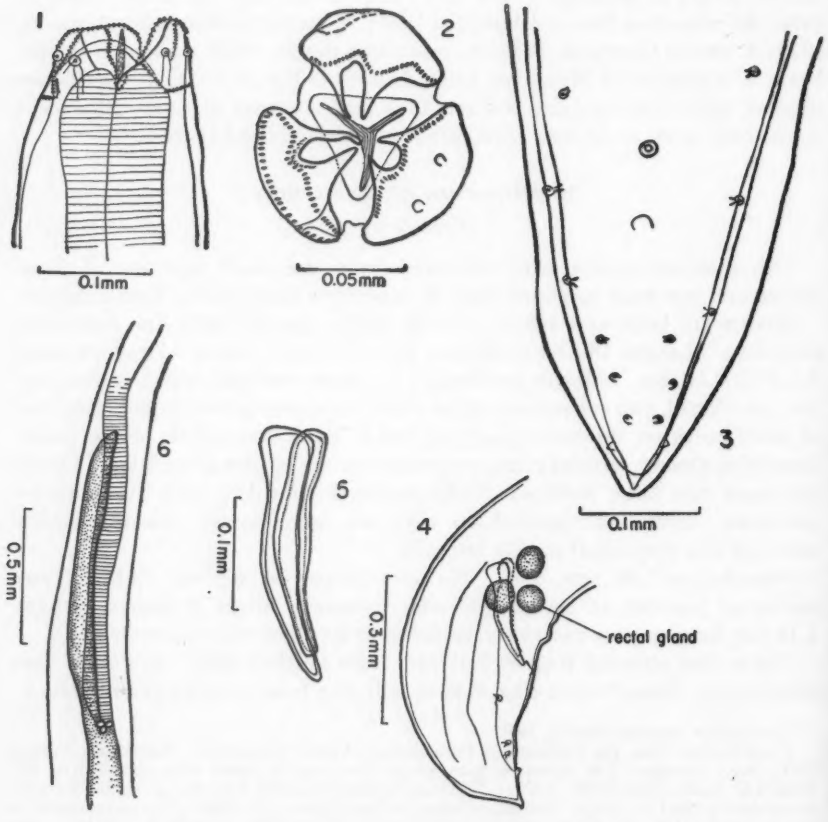
Oesophagus 2.36 mm long. No ventriculus, no distinct bulb. Three valves at junction of oesophagus and intestine. Single intestinal caecum, 1.16 mm long, narrow anteriorly, broadest at its union with intestine.

Nerve ring situated 0.42 to 0.47 mm from anterior end. Excretory pore immediately behind nerve ring, 0.49 to 0.51 mm from anterior end of body.

[1]Manuscript received April 6, 1959.
Contribution from the Institute of Parasitology, McGill University, Macdonald College P.O., Que., Canada. The parasites upon which this paper is based were collected by Dr. Robert E. Kuntz (Cdr. MSC, USN; U.S. Naval Medical Research Unit No. 2, Taipei, Taiwan) a member of the U.S. Naval Medical Mission to East Pakistan in 1958. He was assisted by James Reese, HM, IC, USN, and Charles Knight, HCM, USN, also of NAMRU-2. Dr. Robert F. Inger, Curator of Reptiles, Chicago Natural History Museum, has provided the identifications of the amphibia.
[2]National Research Council of Canada, Postdoctorate Fellow.

Tail short, conical, with terminal spike, 0.23 to 0.24 mm long. Caudal alae much reduced. Five pairs preanal, four pairs postanal caudal papillae. Median papilla on anterior lip of cloaca. Spicules slightly subequal, very short, cylindrical, slightly tapering rods, broader at anterior end, narrower at posterior end. Left spicule 0.2 to 0.22 mm long, right spicule 0.19 to 0.2 mm long. Left spicule narrower than right. Accessory piece absent.

Amplicaecum ranae sp. nov. closely resembles *A. cacopi* Chatterji, 1936 (1) in the number and arrangement of the caudal papillae. It differs from *A. cacopi* in the presence of a median papilla, in the non-possession of well-developed caudal alae, in the absence of interlabia, and in having subequal spicules. In *A. ranae* the spicules are cylindrical, slightly tapering rods,



FIGS. 1–6. *Amplicaecum ranae.*
FIG. 1. Head, dorsal view. FIG. 2. End-on view. FIG. 3. Tail, male, ventral view. FIG. 4. Tail, male, lateral view. FIG. 5. Spicules. FIG. 6. Junction of oesophagus and intestine showing intestinal caecum.

broader at the anterior end and narrower at the posterior end. The left spicule is distinguished from the right in being smaller and narrower. In *A. cacopi* the spicules are identical.

Hosts: *Rana tigrina* and *R. cancrivora.*
Habitat: Small intestine.
Locality: Dacca, East Pakistan.

Reference

1. CHATERJI, R. C. On a new species of Nematoda, *Amplicaecum cacopi* sp. nov. from *Cacopus systosoma.* Ann. Trop. Med. Parasitol. **30**, 41–44 (1936).

THE TAXONOMY OF DIPETALONEMA SPIROCAUDA (LEIDY, 1858) N. COMB. (=SKRJABINARIA SPIROCAUDA) AND DIROFILARIA ROEMERI (LINSTOW, 1905) N. COMB. (=DIPETALONEMA ROEMERI)[1]

ROY C. ANDERSON

Abstract

Filarioid nematodes from *Phoca vitulina* are described and assigned to *Filaria spirocauda* Leidy. Specimens from *Callorhinus ursinus* are close to *F. spirocauda* but their identity remains in doubt. *F. spirocauda* is shown to belong to *Dipetalonema*. Lubimov's (1927) description of *F. spirocauda* as *Skrjabinaria spirocauda* is invalid since his female specimen was a metastrongyle. *Skrjabinaria* falls, therefore, as a synonym of *Dipetalonema*. *Skrjabinaria heteromorpha* is a synonym of *D. spirocauda*. A literature review reveals no authentic record of *Dirofilaria immitis* in seals other than *Zalophus californianus*. *D. fausti* is a synonym of *D. immitis*. *D. spirocauda* (Leidy) n. comb. is similar to *weissi*, *sunci*, and *vite* but the male lacks terminolateral, wing-like appendages and bands of rod-like, cuticular structures on the ventral surface of the caudal extremity. Specimens in the U.S. National Museum (No. 45953) have been chosen as types.

Dirofilaria roemeri (= *Dipetalonema roemeri*) is described from specimens from a kangaroo (probably *Macropus major*) of Queensland, Australia. It differs from other *Dirofilaria* mainly in its unusually small spicules, and its smooth cuticle with lateral alae. The binomial *Filaria websteri* Cobbold, 1879 is regarded as a *nomen nudum*.

A female nematode from the testicular sheath of a fur seal (*Callorhinus ursinus*) from Alaska has recently been sent to the Department of Parasitology by Dr. V. B. Scheffer of the Bureau of Commercial Fisheries, Sand Point Naval Air Station, Seattle, Washington. A preliminary determination of *Skrjabinaria spirocauda* (Leidy, 1858) Lubimov, 1927 was made and further material was requested and obtained from the Agricultural Research Service, Beltsville, Maryland, through the courtesy of Dr. Allan McIntosh and Dr. W. W. Becklund. All the specimens received from the Agricultural Research Service had been identified by members of the Research Service as *S. spirocauda*. A study of these specimens has raised a number of taxonomic problems that it is important to clarify as much as possible. After giving a description of specimens, from the heart of *Phoca vitulina*, which are believed to be conspecific with *Filaria spirocauda* Leidy, 1858, the author will try to show (*a*) that it is not yet possible to conclude that specimens from *Callorhinus ursinus* of the Pacific are conspecific with those from *Phoca vitulina* of the Atlantic, (*b*) that the only detailed description of a female referred to *Filaria spirocauda* pertains, in fact, to a metastrongyle, (*c*) that *Filaria spirocauda* is a species of *Dipetalonema*, and *Skrjabinaria* is a synonym of *Dipetalonema*, (*d*) that *Skrjabinaria heteromorpha* Kreis is a synonym of *Dipetalonema spirocauda* (Leidy, 1858) n. comb., (*e*) that there is no authentic record of *Dirofilaria immitis* in seals other than *Zalophus californianus*, and (*f*) that *Dirofilaria fausti* Skrjabin and Shikhobalova, 1948 is a synonym of *Dirofilaria immitis*.

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Contribution from the Department of Parasitology, Ontario Research Foundation, Toronto 5, Ontario.

In addition to adding *Filaria spirocauda* to the genus *Dipetalonema*, a study of filarioids from a kangaroo has made it possible to remove a species, the taxonomy of which has hitherto been obscure, from this genus. The specimens studied refer to "*Dipetalonema roemeri*" (Linstow, 1905)Yorke and Maplestone, 1926 but they belong to the genus *Dirofilaria* and not *Dipetalonema*. Since no adequate description of *Dirofilaria roemeri* (Linstow, 1905) n. comb. exists, the material, sent from Australia by Dr. J. C. Pearson of the Veterinary School, Yeerongpilly, is described in detail and the species is compared with other members of the genus.

1. *Dipetalonema spirocauda* (Leidy, 1858) n. comb.

(Figs. 1–3, 8–9)

Filaria spirocauda Leidy, 1858, 112 (*Phoca vitulina*)(16); Railliet, 1899, 130 (*P. vitulina*)(20)

Skrjabinaria spirocauda (Leidy) Lubimov, 1927, not female, 305, Figs. 12–15 (*Phoca vitulina*)(18); Skrjabin and Shikhobalova, 1948, not female, 160, Fig. 36 (23).

Skrjabinaria heteromorpha Kreis, 1953, 108–113, Figs. *a–f* (*Phoca vitulina*)(15)

Descriptive Account

Description.—Onchocercidae; Dipetalonematinae; *Dipetalonema* Diesing, 1861. Long, slender nematodes with gently tapering extremities. Anterior end rounded without raised, cuticular structures, bearing four pairs of submedian papillae and lateral, well-defined amphids. Oral opening surrounded by cuticular ring leading into well-developed buccal cavity with thick, curved, refractory wall. Wall of buccal cavity and oesophagus separated by thick, cuticular ring. Oesophagus rather short, clearly divided into short, anterior, muscular part and broader, posterior, glandular part. Posterior end of oesophagus club-shaped. Cuticle thin, often with obscure, regular, transverse striations, composed of criss-crossing, diagonally running fibers, slightly thickened on lateral sides. Excretory pore generally distinct in females, not observed in males. Deirids not observed.

Male.—(Two specimens (1) U.S.N.M. No. 45953 (neotype by subsequent designation); (2) U.S.N.M. No. 4436.) Length 87, 93 mm. Maximum width 0.40, 0.33 mm, near middle of body. Caudal end with three to four coils. Nerve ring ill-defined, 0.34 mm from anterior extremity. Oesophagus 1.9, 2.0 mm in length; muscular part 0.58, — mm in length. Caudal extremity with narrow, highly inconspicuous, lateral alae. Tail 0.23, 0.20 mm in length, narrow, rounded, terminating in four, often obscure papillae (Fig. 1, No. 4436). Anus surrounded by slightly inflated cuticle. Caudal papillae sessile, arranged as follows (cf. Fig. 1, No. 4436; Fig. 3, No. 45953): (*a*) three to four pairs of preanal papillae; (*b*) five smaller papillae in row immediately behind anus, the two outermost being largest; (c) an unpaired papilla on left side of ventral surface about 80 μ behind anus (unpaired in No. 4436 but perhaps paired in No. 45953); (*d*) a single, broad, median papilla immediately before anus;

(*e*) a pair of papillae on the ventrolateral surface about 15 μ from caudal extremity; (*f*) two pairs of terminal papillae already mentioned. Left spicule 0.47, – mm in length (damaged in No. 4436) consisting of tubular calomus 0.19, – mm in length and well-developed, partially membranous lamina about 0.28, – mm in length. Filament not recognizable. Right spicule 0.21, 0.27 mm in length, consisting of tubular calomus about 0.10 mm in length, and broad, spatulate lamina. Capitula of spicules weakly developed and gubernaculum absent. Ventral surface of caudal extremity devoid of rod-like cuticular formations.

Female.—(Two specimens (1) U.S.N.M. No. 45953 (type by subsequent designation); (2) U.S.N.M. No. 4436). Length 155, 143 mm. Maximum width 0.66 mm, near middle of body. Nerve ring 0.33, 0.38 mm from anterior extremity. Excretory pore 0.45, – mm from anterior extremity. Oesophagus 1.3, 1.8 mm in length, muscular part 0.55, – mm in length. Vulva, 1.2, 1.3 mm from anterior extremity, guarded by two lateral, rounded flaps. Anterior part of vagina sharply bent and bound with ligament as typical of *Dipetalonema*. Total length of vagina not determinable because of convolutions. Tail 0.36, 0.25 mm in length rounded and terminating in pair of well-defined phasmids (Fig. 2, No. 45953).

Host.—Phoca vitulina concolor Dekay, 1842.

Location.—No. 45953, pulmonary artery and right ventricle.
No. 4435, heart.

Locality.—The coast of Maine, U.S.A.

Specimens.—Nos. 45953 (types by subsequent designation), 4436, United States National Museum.

Other specimens.—D. ? spirocauda from *Callorhinus ursinus* (Linné, 1758) (Figs. 12, 14–16.) The collection consists of four females and one male.

Male: (U.S.N.M. No. 40100.) Dimensions: Length 53 mm. Maximum width 0.19 mm. Muscular oesophagus 0.58 mm, glandular 1.2 mm in length. Nerve ring 0.28 mm from anterior extremity. Right spicule 0.22 mm; left 0.60 mm in length. Tail 0.23 mm in length. Morphology agreeing in most particulars (e.g. head, spicules) with specimens from *Phoca vitulina*. Specimen surrounded by loose cuticle, presumably the result of molt, giving caudal end more pointed appearance. Papillae as in Fig. 1 as judged from study in lateral view except that the more anterior preanal papillae (two on the right side and three on the left) are further from the anus (Fig. 16).

Females: (U.S.N.M. Nos. 40100, 33073, 45678; Nematode Collection Ontario Research Foundation No. 28.) Dimensions: Length 170, 113, 139, 106 mm. Maximum width 0.32, 0.33, 0.45, 0.45 mm. Muscular oesophagus 0.51, 0.41, 0.54, 0.50 mm, glandular 1.1, 1.1, 1.0, 1.0 mm. Nerve ring 0.26, 0.23, 0.34, 0.22 mm. Vulva 1.1, 1.0, 1.3, 0.86 mm. Excretory pore 0.53, 0.59, 0.37 mm. Tail 0.27, 0.17, 0.22, 0.23 mm. Morphology agreeing in all particulars with specimens from *Phoca vitulina* except that tail is broader and terminates in three cuticular protuberances (Figs. 14–15).

Microfilaria (from uterus of specimen No. 28): Exceedingly slender with greatly attenuated caudal extremity. Length 10 microfilariae preserved in alcohol and cleared in glycerine 185–235 (215) μ. Maximum width 4–4.5 μ. No sheath observed.

Location: Ontario Research Foundation No. 28—testicular sheath.
U.S.N.M. No. 40100—subcutaneous tissue.
U.S.N.M. No. 33073—subcutaneous tissue.
U.S.N.M. No. 45678—upper colon.

Locality: Alaska.

Comments

None of the above specimens from *Callorhinus ursinus* is in good condition. The general similarity in the dimensions and morphology of the buccal cavity, spicules, and oesophagus with the specimens from *Phoca vitulina* is striking. Nevertheless, for the following reasons the author is reluctant to conclude definitely at this time that they belong to the same species: (*a*) except for specimen U.S.N.M. No. 45678, which was found in the colon, the others are from subcutaneous tissues instead of the heart or major blood vessels; (*b*) the geographic distribution differs; (*c*) the tip of the female tail, although in poor condition in some specimens, ends in three small, cuticular elevations. The apex of the male tail is distorted and covered with loose cuticle and debris but it appears to be more pointed (and possibly trifid) than in specimens from *Phoca vitulina* (Fig. 16); (*d*) the larger preanal male papillae are considerably more anterior to the anus than their counterparts in the specimens from *Phoca vitulina* (Fig. 16).

One suspects that *S. spirocauda* may be a widely distributed and highly variable species that can occur in various regions of the host but a decision must be suspended until new material from the Pacific becomes available.

The taxonomy of *Dipetalonema spirocauda* has been greatly complicated by the existence in seals of another filarioid, *Dirofilaria immitis*, and by the fact that most of the earlier descriptions are so summary as to make it impossible to determine the species to which they refer. In addition, the only modern description of a female under the name *D. spirocauda*, that of Lubimov, 1927, is not of a filarioid.

Leidy's (16) description of *Filaria spirocauda* from the heart of *Phoca vitulina* was as follows: "Body long, filiform, most narrowed posteriorly. Head obtuse, unarmed, mouth a minute, circular pore, neither armed nor labiated. Tail short, conical, rather obtuse, or subacute; anus just above the tail. Caudal extremity of female recurved, of male wound into a spiral of three to four turns. Length of female 6 to 8 inches, breadth $\frac{1}{2}$ of a line; length of male 4 inches, breadth $\frac{1}{3}$ of a line." The type specimens of *F. spirocauda* have evidently been lost (8).

In the same year Joly (14) described as *Filaria cordis phocae* a single female from the heart of *Phoca vitulina* as follows: "Corps blanchâtre, filiforme, long de 15–20 centimètres, atténué et recourbé en crochet à sa partie postériure.

Tête obtuse, sans papilles. Bouche nulle. Anus nul. Vulve? Tégumen finement strié en travers, présentant au microscope des fibres entre-croisées à la manière de celles des *Mermis*, et recouvrant un tube interieur, formé de fibres ou de lames longitudinales. Oeufs elliptique. Male inconnu."

Cobbold (5) named a worm from the heart of *Stemmatopus cristatus* as *Filaria hebetata* but in 1879 (6, p. 314) he considered it as belonging to *Filaria spirocauda*.

Railliet (20) described but, unfortunately, did not illustrate specimens from *Phoca vitulina* that he called *Filaria spirocauda*. The following features of his description seem significant: "Deux spicules inegaux, l'un de 700 μ, l'autre de 350 μ . . ." and vulva " . . . à 1 mm.–1.3 mm. de l'extremité cephalique . . .".

Lubimov (18) described in detail filarial worms from the heart of *Phoca vitulina*, which he referred to Leidy's species, and for which he erected the new genus *Skrjabinaria*. This description, regarded as the type description of *Filaria spirocauda*, contains a serious error which it will be necessary to point out further on.

Faust (8) described worms from the heart of a California sea lion (*Zalophus californianus*) which were indistinguishable from the types of *Dirofilaria immitis* (Leidy, 1856), the latter having been recovered from the domestic dog in the United States. Faust considered that his material and that of Railliet (20) from *Phoca vitulina* were probably conspecific.

Bisbocci (3) reported the existence of *Dirofilaria immitis* in the heart of a *Phoca vitulina* from Hamburg that was maintained for a time in Torino. He did not illustrate his specimens (one male and three females) but gave a short description with certain dimensions.

Skrjabin and Shikhobalova (23) reproduced Faust's description of *Dirofilaria immitis* from *Zalophus californianus* and, apparently unaware that Faust mentioned in a footnote that he had compared his material with Leidy's types of *D. immitis* and found them indistinguishable, proposed for them the new name *Dirofilaria fausti*.

Kreis (15) described *Skrjabinaria heteromorpha* from the heart of *Phoca vitulina* and claimed that it differed from *S. spirocauda*, as described by Lubimov (1927), in the presence of tooth-like, cuticular thickenings at the anterior end of the oesophagus, in the ratio of the two parts of the oesophagus, the position of the vulva, and in the possession of four pairs of preanal and five pairs of postanal papillae.

A careful consideration of the above descriptions and reports of filarioids from the hearts and blood vessels of seals leads to a number of important conclusions. The descriptions of Leidy (16) and Joly (14) are so general that they could apply to either *Dirofilaria immitis* or *Dipetalonema spirocauda* (as determined by Lubimov's description of the male). Since no description accompanies the name *Filaria hebetata*, there is no way of knowing the species to which it refers and it cannot, therefore, be regarded as a synonym of either *D. immitis* or *D. spirocauda*. The earliest description which contains elements sufficient to indicate one or other of the two species is that of Railliet (20),

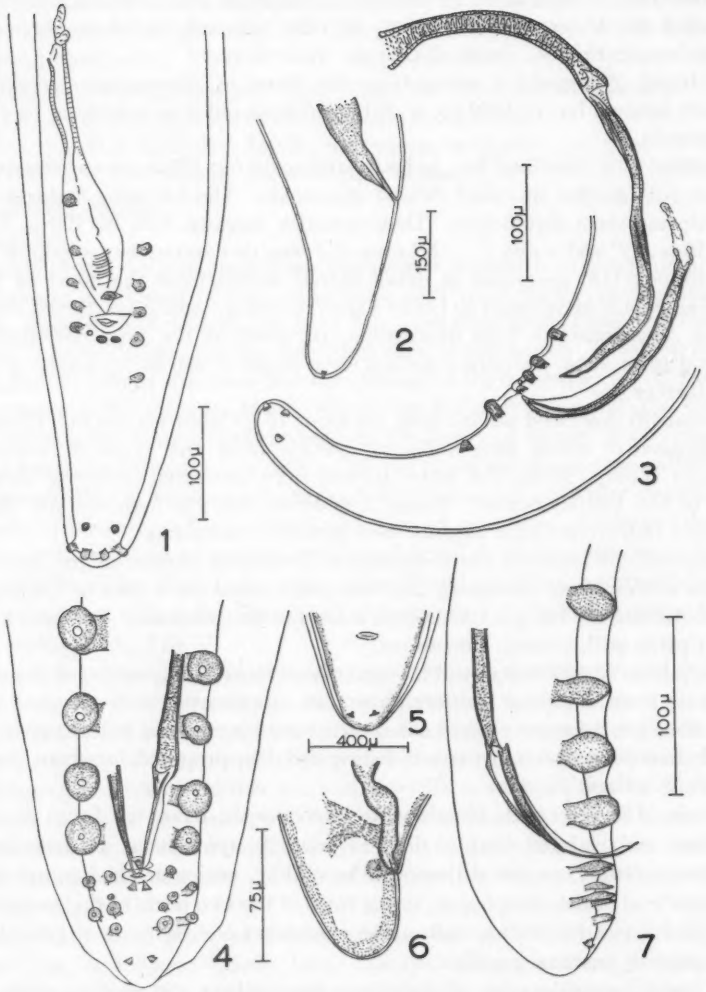

FIG. 1. *Dipetalonema spirocauda*, caudal end male, ventral view (U.S.N.M. No. 4436).
FIG. 2. *D. spirocauda*, caudal end female, lateral view (U.S.N.M. No. 45953).
FIG. 3. *D. spirocauda*, caudal end male, lateral view (U.S.N.M. No. 45953).
FIG. 4. *Dirofilaria roemeri*, caudal end male, ventral view.
FIG. 5. *D. roemeri*, caudal end female, ventral view.
FIG. 6. *D. roemeri*, caudal end female, lateral view.
FIG. 7. *D. roemeri*, caudal end male, lateral view.

who gives the lengths of the spicules as 350 μ and 700 μ and the position of the vulva as 1.0–1.3 mm. These data agree closely with Lubimov's description of the male which he assigned to Leidy's species (*spirocauda*) as well as the material described herein.

Many features of Bisbocci's (3) description of his specimens from *P. vitulina* agree with the material described herein. For example, he refers to the buccal aperture as being 21 μ in size ("l'apertura boccale di μ 21"), the male tail as 0.28 mm in length, narrow, and with weakly developed alae. There is nothing in his description of the female which does not accord with the specimens from *Phoca vitulina* in North America. He gives the lengths of the spicules as 0.20 mm (right) and 0.29 mm (left), however, which agrees more with *D. immitis* than *D. spirocauda*. It is not possible to decide, therefore, which of the two species he may have had.

This review of the literature indicates that Faust's report (8) is the only authentic record of *Dirofilaria* in Pinnipedia. Since *D. immitis* occurs in dogs in California (22) and is most prevalent in the coastal areas (at least this has been shown in the eastern U.S.A. (19)), there is little reason for doubting the validity of this diagnosis. Indeed, the discovery of *D. immitis* in *Z. californianus* may support Otto's (19) suggestion that brackish water mosquitoes are involved in the life cycle. Like certain other filarial worms (e.g. *Brugia malayi*, *Aproctella stoddardi*, *Pseudaproctella inornata*), *D. immitis* exhibits a considerable lack of host specificity, having already been reported from primates, rodents, cats as well as dogs, wolves, and foxes (1). For these reasons *Dirofilaria fausti* is regarded as a synonym of *D. immitis*.

Since there is no authentic record of *D. immitis* in the harbor seal, Lubimov's (18) decision to assign his specimens to *Filaria spirocauda*, even though Leidy's original description is inadequate, seems sound. His description is, therefore, extremely important since, apart from Railliet's (20) brief comments, it is the only description of nematodes from the heart of seals which clearly does not refer to *D. immitis*.

Lubimov's description of the male agrees closely with the male specimens from *Phoca vitulina* studied herein. He gives the following data: Size 140×0.669 mm. Nerve ring 0.397. Oesophagus 1.86×0.13 mm. Anus 1.67 mm (presumably 0.167 mm). Tail end spiralled 3 to 4 times with three pairs of preanal papillae and four pairs of postanals (he did not study his specimen in ventral view). His description of the spicules is exceedingly close to that observed herein although the left had a length of 0.648 mm (cf. Railliet (20), who gives it as 700 μ); the right spicule measured 0.243 mm. Moreover, his lateral view of the anterior extremity of the male with its two pairs of submedian papillae (one side only), as well as the oesophagus, leaves little doubt that the specimens described herein from *Phoca vitulina* are conspecific with it. It is on this basis that the present material is assigned to *Filaria spirocauda*.

On the other hand, the morphology of Lubimov's female specimen differed so radically from the male that undoubtedly it did not belong to the same species. His description of the first-stage larvae (length 0.324×0.027 mm in

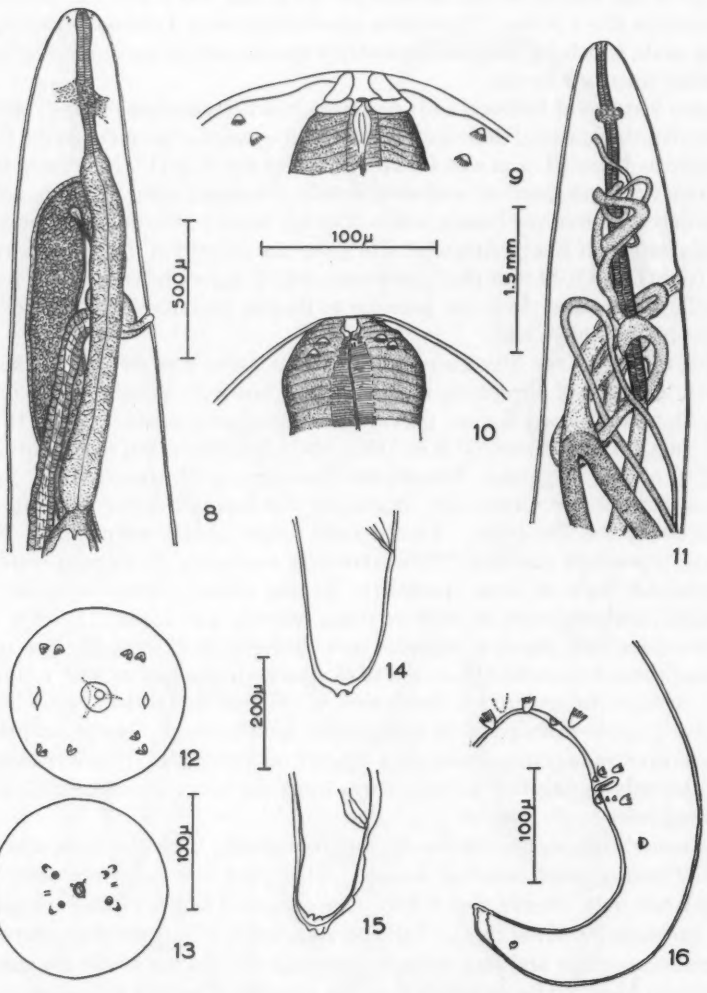

FIG. 8. *Dipetalonema spirocauda*, anterior end female, lateral view (U.S.N.M. No. 45953).
FIG. 9. *D. spirocauda*, anterior end female (U.S.N.M. 4436).
FIG. 10. *Dirofilaria roemeri*, anterior end male, lateral view.
FIG. 11. *D. roemeri*, anterior end female, lateral view.
FIG. 12. *Dipetalonema ? spirocauda*, anterior end female, *en face* view (O.R.F. No. 28).
FIG. 13. *Dirofilaria roemeri*, anterior end male, *en face* view.
FIG. 14. *Dipetalonema ? spirocauda*, caudal end female, lateral view (U.S.N.M. 45678).
FIG. 15. *D. ? spirocauda*, caudal end female, lateral view (U.S.N.M. No. 33073).
FIG. 16. *D. ? spirocauda*, caudal end male, lateral view (U.S.N.M. No. 40100).

size, with a tooth-like structure, an oesophagus 0.078–0.080 mm in length and an undulating tail 0.048 mm in length), the short, club-shaped, undivided oesophagus, the short, cone-shaped tail, the cephalic extremity with six lip-like structures and six papillae, and the existence of a teguminal sheath indicates clearly that his female specimen was a metastrongyle. It is not possible to assign this female to any known metastrongyle, especially since the vulva was said to be 28 mm from the cephalic extremity. If this latter measurement was a mistake, however, one would suspect strongly that the specimen belonged to *Parafilaroides gymnurus* (Railliet, 1899) Dougherty, 1946, which is a parasite of *Phoca vitulina*.

The male described by Lubimov, as well as all the specimens described herein, belong to *Dipetalonema* as conceived by Chabaud (4). Unfortunately, since Lubimov did not describe a new species, he naturally did not choose a holotype and it is difficult to know what to do with the genus *Skrjabinaria*, which is based on two species. He described his male specimen first and in more detail than the female and conceivably he regarded it as the most important. On the other hand, helminthologists, in differentiating *Skrjabinaria* from other filarioids, have naturally stressed the cephalic characteristics of the female metastrongyle. As a somewhat arbitrary solution to this problem, the author suggests that most importance be attached to Lubimov's description of the male and that *Skrjabinaria* be placed as a synonym of *Dipetalonema*. If new material shows that Lubimov's female specimen belonged to an undescribed genus then a new name can be proposed.

As noted previously the type specimens of *Filaria spirocauda* Leidy, 1858 have been lost. In an attempt to avoid further confusion in the taxonomy of this species, the author has chosen as types by subsequent designation specimens No. 45953 in the United States National Museum.

The validity of *Skrjabinaria heteromorpha* seems highly doubtful on theoretical grounds, since one would have to accept the existence of two closely related filarioids of the same genus in the same host. Moreover, the tooth-like projections mentioned by Kreis (15) are undoubtedly the cuticle of the oesophagus which has been forced upward during molting (this has been noted in male specimen No. 40100). The rather minor differences noted by Kreis in the oesophagus, position of the vulva, and the arrangement and number of the caudal papillae are easily explained by the well-known variability of these structures in filarioids. *S. heteromorpha* is regarded, therefore, as a synonym of *D. spirocauda*.

A comparison of *D. spirocauda* with others in the genus indicates that it is a valid species. Only three other species of *Dipetalonema* are reported from carnivores, namely *reconditum*, which possesses a neck-like constriction behind the head end and a swelling in the cervical region; *grassii*, in which the microfilaria is extremely large (567 $\mu \times 12$ μ); and *dracunculoides*, which is evidently a much smaller species. In the form of its spicules, oesophagus, anterior end, buccal cavity, and the position of its vulva, *spirocauda* seems to be morphologically closest to *vite* (=*blanci*), *weissi*, and *sunci* of rodents and

insectivores. However, the tails of the males of both *weissi* and *sunci* end in a terminal point and two lateral wing-like appendages, the deirids of *weissi* are highly developed, and *sunci* possesses only a single pair of preanal papillae. The spicules, oesophagus, and buccal cavity, as well as the arrangement of the caudal papillae, are remarkably similar in *spirocauda* and *vite* but the former lacks bands of cuticular rods on the ventral surface of the caudal extremity and many of the important dimensions are different, e.g. spicules.

2. *Dirofilaria roemeri* (Linstow, 1905) n. comb.

(Figs. 4–7, 10–11, 13)

Filaria roemeri Linstow, 1905, 356–358, Pl. 23, Fig. 2 (*Macropus antilopinus*) (17)

Acanthocheilonema roemeri (Linstow) Baylis, 1925, 114–115 (*Macropus giganteus*) (2)

Dipetalonema roemeri (Linstow) Yorke & Maplestone, 1926, 429 (24); Johnston and Mawson, 1938, 111–112, Figs. 8–13 (*Macropus major*, *M. robustus*, *M. melanops*, *M. dorsalis*, *M. parryi*, *M. ruficollis*, *Onychogales frenata*) (9)—1939, 309 (*Macropus giganteus*) (10)—1940, 369 (11)—1940, 365 (12)—1949, 69 (*Macropus ocydromus*, *M. agilis*) (13)

Descriptive Account

Description.—Onchocercidae; Dirofilariinae; *Dirofilaria* Railliet and Henry, 1911. Large, broad nematodes, especially the female, tapering markedly at extremities. Anterior extremity rounded without raised, cuticular structures, bearing four pairs of submedian papillae and lateral amphids. Stoma surrounded by delicate, cuticular ring leading into minute, cylindrical, buccal capsule as broad as long with delicate, refractory walls. Oesophagus narrow, not clearly divided into two portions, slightly bulbous anteriorly and surrounding delicate, buccal capsule. Cuticle with regular, transverse striations composed of longitudinal and diagonal fibers; longitudinal ridges absent but pair of lateral alae present extending most of length of body. Excretory pore and deirids not observed.

Male.—Length 32 mm. Maximum width 0.66 mm, near middle of body. Nerve ring 0.29 mm from anterior extremity. Oesophagus 1.5 mm in length; muscular portion perhaps about 0.69 mm in length but junction between two parts of oesophagus not clear. Caudal extremity with broad, lateral alae up to 2.0 mm in length. Tail short, rounded, 0.07 mm in length. Anus surrounded by cuticular ring. Caudal papillae as follows: (1) four pairs of large pedunculate preanals; (2) six pairs of irregularly arranged, smaller, pedunculate papillae on the ventrolateral surface of the tail, the first pair on a level with the anus; (3) a single broad, sessile, median papilla immediately in front of cuticular ring surrounding anus, the apex of which is directed posteriorly; (4) a pair of sessile papillae immediately behind anus, the apices of which are directed anteriorly. Phasmids on ventral surface of tail about 17 μ from caudal extremity. A single postdeirid is present on the left side 1.4 mm

from caudal extremity (careful examination failed to reveal another). Left spicule 0.17 mm in length consisting of a tubular calomus 65 μ in length, an alate lamina 95 μ in length, and a short, delicate filament 10 μ in length. Right spicule 72 μ in length, markedly tapering. Neither spicule with capitulum, and gubernaculum absent.

Female.—Length 145 mm. Maximum width 2.0 mm, near middle of body. Cuticle transparent, the alimentary and genital tracts visible with naked eye in uncleared specimen. Indistinct nerve ring 0.90 mm from anterior extremity. Oesophagus narrow, 3.8 mm in length, much obscured by coils of genital system. Vulva on slight swelling 2.6 mm from anterior extremity. Length of vagina not determined because of convolutions. Caudal end rounded, provided with delicate ventrolateral phasmids. Anus obscure, 0.33 mm from caudal extremity, surrounded by exceedingly delicate, cuticular ring.

Microfilaria (in uterus of female 20 mm from cephalic extremity).—Small and slender with delicate sheath extending over extremities. Length five specimens preserved in formalin 190–218 (204) μ. Maximum width 5–6 μ. Nerve ring 16%, excretory pore 35%, and anal pore 81% approximately of body length from anterior extremity.

Host.—Kangaroo, probably *Macropus major.*

Location.—Subcutaneous tissues.

Locality.—Thargominda, Queensland, Australia.

Specimens.—Nematode Collection, Ontario Research Foundation No. 27.

Comments

It is perhaps worth noting that a few microfilariae were found in the intestine of the male specimen. The microfilariae in the anterior part of the intestine had the appearance of those found in the uteri of the female but further down they appeared degenerate suggesting that they had been partially digested.

Previous descriptions of this species have been extremely summary but the data given by Linstow (17), Baylis (2), and especially Johnston and Mawson (9) show conclusively that these authors were dealing with a species of *Dirofilaria* and not *Dipetalonema* (= *Acanthocheilonema*). The specimens described herein apparently belong to the same species, which is, according to various articles by Johnston and Mawson, common in kangaroos. The present material agrees well with previous descriptions (9, 17). It is remarkable that *D. roemeri*, in spite of these earlier descriptions, should never have been placed in the correct genus.

Skrjabin and Shikhobalova (23) reproduced the figures and description of Linstow (17) and Johnston and Mawson (9) under the name *Dipetalonema websteri* (Cobbold, 1879). This latter name was proposed by Cobbold (5) for the combination *Filaria macropi majoris* found in the catalogue of the Royal College of Surgeons, London (1830–37), and said to refer to worms from the knee joint of a kangaroo. Diesing (7) apparently altered the name to *F. macropodis gigantei.* Railliet and Henry (21) suggested it might refer to *Onchocerca.* Yorke and Maplestone (24) placed it under *Dirofilaria.* In

spite of these, and other references already noted by Johnston and Mawson (9), no description of the original material exists. In addition, Baylis (2) reported that the material referred to in the catalogue of the Royal College of Surgeons was in such poor condition that its identity to *Filaria roemeri* could not be established. Clearly, therefore, *Filaria websteri* is a *nomen nudum* and there is no justification for its use.

Although the genus *Dirofilaria* is in need of a thorough revision, *D. roemeri* (Linstow, 1905) n. comb. is so distinct that it can be separated readily from the other species in the genus. As far as can be determined no species of *Dirofilaria* has been reported to possess lateral alae. The absence of longitudinal ridges on the cuticle separates *D. roemeri* readily from *acutiuscula*, *conjunctivae*, *repens*, *spectans*, *subdermata* (=*subcutanea*, = *spinosa*, = *hystrix*), *tenuis*, and *ursi* (= *desportesi*). The cuticle of *D. immitis* (= *fausti*) lacks longitudinal ridges but lateral alae are absent as well. In many species the spicules are much longer, e.g. the left spicule is over 400 μ in length, more than double that of *D. roemeri*, in *incrassata*, *corynodes* (= *shoutedeni*, = *aethiops*), *granulosa*, and *macacae*. The differences between *D. roemeri* and the remaining species are only slightly less extreme. In *sudanensis* (of which *minor* and *pagumae* are probably synonyms), *freitasi*, and *kuelzi* (= *asymmetrica*) the right spicule is twice as long as in *roemeri* and the number and distribution of the caudal papillae are different; the anus in the female of *kuelzi* is subterminal as well. Although the size of the spicules of *D. scapiceps* is closer to that of *roemeri*, it is an unusually small species (male 12–14.5 mm; female 25–30 mm). *D. bonnei*, the caudal end of the male of which is undescribed, probably does not pertain to *Dirofilaria* since the tail of the female is so long in relation to the width of the body; this species may be a member of the genus *Dipetalonema*.

Dirofilaria roemeri is the only species of the genus reported from Australian marsupials.

Acknowledgments

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References

1. ANDERSON, R. C. Description and relationships of *Dirofilaria ursi* Yamaguti 1941 and a review of the genus *Dirofilaria* Railliet and Henry, 1911. Trans. Roy. Can. Inst. **29**, 35–65 (1952).
2. BAYLIS, H. A. Notes on some Australian parasitic nematodes. Ann. Mag. Nat. Hist. Ser. 9, **15**, 112–115 (1925).

3. BISBOCCI, G. Di un caso di parassitismo da *Dirofilaria immitis* Leidy in una *Phoca vitulina*. Riv. parassitol. **3**, 113–115 (1939).
4. CHABAUD, A. G. Le genre *Dipetalonema* Diesing 1861; essai de classification. Ann. parasitol. humaine et comparée, **27**, 250–285 (1952).
5. COBBOLD, T. S. Observations on haematozoa. Veterinarian, **46**, 674–677 (1873).
6. COBBOLD, T. S. Parasites; a treatise on the entozoa of man and animals, including some account of the ectozoa. London. 1879.
7. DIESING, K. M. Systema helminthum. Vol. 2. Vindobonae (1851).
8. FAUST, E. C. Mammalian heart worms of the genus *Dirofilaria*. Festschr. Bernhard Nocht zum, **80**, Geburtstag, Hamburg, 131–139 (1937).
9. JOHNSTON, T. H. and MAWSON, P. M. An account of some filarial parasites of Australia marsupials. Trans. Roy. Soc. S. Australia, **62**, 107–121 (1938).
10. JOHNSTON, T. H. and MAWSON, P. M. Some nematodes from Victorian and western Australia marsupials. Trans. Roy. Soc. S. Australia, **63**, 307–310 (1939).
11. JOHNSTON, T. H. and MAWSON, P. M. A key to the nematode parasites of Australian marsupials and monotremes. Trans. Roy. Soc. S. Australia, **64**, 363–370 (1940).
12. JOHNSTON, T. H. and MAWSON, P. M. On a collection of nematodes from Australian marsupials. Records Australian Museum, **20**, 360–366 (1940).
13. JOHNSTON, T. H. and MAWSON, P. M. Some nematodes from Australian hosts, together with a note on *Rhabditis allgeni*. Trans. Roy. Soc. S. Australia, **73**, 63–71 (1949).
14. JOLY, N. Sur une nouvelle espèce d'hematozoaire du genre *Filaria* (*Filaria cordis phocae* N. Joly), trouvé dans le coeur d'un phoque. Mém. acad. sci. Toulouse, **2**, 166–180 (1858).
15. KREIS, H. A. Beiträge zur Kenntnis parasitischer Nematoden. XIII. *Skrjabinaria heteromorpha* n. sp. (Filarioidea Weinland, 1858; Stiles, 1907) aus dem Seehund (*Phoca vitulina* L.). Zool. Garten, Leipzig, **20**, 108–113 (1953).
16. LEIDY, T. Contributions to helminthology. Proc. Acad. Nat. Sci. Phila. **10**, 110–112 (1858).
17. LINSTOW, O. F. B. Helminthologische Beobachtungen. Arch. Mikrobiol. Anat. **66**, 355–366 (1905).
18. LUBIMOV, M. P. (Concerning filariae found in the animals of the Zoopark of Moscow.) [Russian text.] Trudy Lab. Eksper. Biol. Mosk. Zooparka, **3**, 295–311 (1927).
19. OTTO, G. F. Heartworm in dogs. North Am. Veterinarian, **30**, 181–189 (1949).
20. RAILLIET, A. Sur quelques parasites rencontrés à l'autopsie d'un phoque (*Phoca vitulina* L.). Compt. rend. soc. biol. Paris, **51**, 128–130 (1899).
21. RAILLIET, A. and HENRY, A. Les onchocerques, nématodes parasites des tissu conjunctif. Compt. rend. soc. biol. Paris, **68**, 248–251 (1910).
22. ROBERTS, I. M. and ROBERTS, S. R. Canine filariasis—a report. J. Am. Vet. Med. Assoc. **109**, 490 (1946).
23. SKRJABIN, K. I. and SHIKHOBALOVA, N. P. (Filariae of animals and man.) [Russian text.] Moscow. 1948.
24. YORKE, W. and MAPLESTONE, P. A. The nematode parasites of vertebrates. J. & A. Churchill Ltd., London. 1926.

THE SALIVARY GLAND CHROMOSOMES OF SEVEN FORMS OF BLACK FLIES INCLUDED IN EUSIMULIUM AUREUM FRIES[1]

ROBERT W. DUNBAR

Abstract

Seven closely related cytological forms are known for the dichromosomic black fly *E. "aureum"*. All these forms have extremely similar salivary gland chromosomes but they differ from each other by (1) the floating (intraspecific) rearrangements present, (2) the details of the sex-determining mechanisms, and (3) at least one homozygous (interspecific) inversion in the first chromosome. Six of these forms are accounted for as three sympatric sibling species pairs. Although certain allopatric forms may yet be identified as geographic variants of a polymorphic species, evidence is presented to show that all seven forms are biologically distinct species. Their phylogenetic interrelationships have been traced by means of the homozygous interspecific inversions.

Introduction

The holarctic black fly species *Eusimulium aureum* Fries apparently consists of a complex of siblings species. In southern Ontario two sympatric siblings have been described (8) which differ in the basic banding sequence of the salivary gland chromosomes, in the floating inversions present, and in the sex-determining mechanisms. These siblings do not interbreed as shown by (1) the failure to find critical heterozygous loops for the interspecific inversions, (2) the absence of shared floating inversions, and (3) the maintenance of individual sex-determining mechanisms. In this paper the information on the previously recognized siblings, A and B, is extended and five additional forms are described, two from Churchill, Manitoba, two from Leningrad, U.S.S.R., and one from California. These forms also differ in important aspects of their cytology similar to those which distinguish the Ontario siblings.

Materials

Taxonomic history, distribution, and habitat of *Eusimulium aureum* Fries were briefly reviewed in the previous paper (8) and data on collecting sites for siblings A and B near Toronto and Montreal were given. Meanwhile further collections were made of both A and B from the *Holland Marsh** in the Toronto area (Table I). Collections made in the Gatineau Hills in Quebec by Lewis Davies in 1958 were entirely of form A. These collecting sites, about 20 miles north of Ottawa, Ontario, are (1) *Breckenridge, South*, (2) *Mulvihill Road* at bottom stream, (3) stream at Ramsay Lake, and (4) *Notch Road* at Mulvihill Stream (Table I). A few specimens of form B from a stream running through *Dayville*, Grant County, Oregon, were received from D. M. Wood (Table I).

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*Place names in italics refer to principal collecting sites.

TABLE I

Collection data for siblings A and B showing sample size, location, and date (location totals are shown in parentheses)

Location	Collection date	Frequency of sibling A	Frequency of sibling B
Toronto area*			
Dixie Creek	1952, 1954	(8)	(33)
Dentonia Park	1953	(8)	(3)
Terra Cotta	1953, 1956	(62)	(51)
N. of Terra Cotta	1956		(4)
Alliston Cutoff	1956		(1)
Highway 400	1956	(49)	(167)
Holland Marsh	1956	82	176
	June 22, 1957	4	34
	June 16, 1958	7	2
		(93)	(212)
Montreal area*			
Montreal, Que	1953, 1954	(2)	(7)
Prefontaine, Que.	1953	(2)	
Ottawa area			
Breckenridge, S.	June 16, 1958	(1)	
Ramsay Lake	July 3, 1958	3	
	July 14, 1958	2	
	Aug. 6, 1958	2	
		(7)	
Mulvihill Brook	July 7, 1958	10	
	July 14, 1958	9	
	July 28, 1958	13	
	Aug. 6, 1958	15	
	Aug. 25, 1958	33	
		(80)	
Notch Road	Aug. 6, 1958	(4)	
Dayville, Oregon	June 17, 1958		(3)

*For details prior to 1957 see (8), Table I.

Collections of *E. "aureum"* from Churchill, Manitoba, were of siblings C and D. They came from only three streams (Table II). Larvae of sibling C were taken during four summers from a short *coastal trickle* running through a tundra meadow. It apparently drained two small tundra pools on the first ridge, 2.5 miles east of the army camp. In 1957 larvae of C were also found in the first large *coastal stream* about 200 yards on the road past the rocket-launching site. Sibling D was found during three summers in a tributary of *Warkworth Creek* in the taiga 0.6 miles east of the section house north of Digges, on the Hudson Bay Railroad.

These last two collecting sites are quite similar in nature. Both streams had flat, clean sandy or gravelly bottoms from which grew principally a sedge, *Carex acuta* L., in the taiga stream, and *Carex* sp. in the coastal stream. It was on the leaves of these that the larvae of *E. "aureum"* siblings could be found most frequently. The banks of both streams were lined with thick

TABLE II

Collection data for siblings C and D (see Table I)

Location	Collection date	Frequency of sibling C	Frequency of sibling D
Churchill area			
Coastal trickle	Aug. 16, 1951	6	
	July 31, 1953	8	
	Aug. 6, 1953	16	
	Aug. 18, 1953	5	
	July 22, 1955	7	
		(42)	
Coastal stream	Aug. 1, 1957	17	
	Aug. 2, 1957	104	
		(121)	
Warkworth Creek	June 7, 1951		1
	July 1, 1955		8
	July 11, 1956		68
	July 15, 1956		131
			(208)

scrubby willow brush which is very common along the streams in the area. The vegetation at the taiga site was somewhat more luxuriant owing to the protection of the trees. The two streams were separated by 10 miles of flat transitional land between taiga and tundra where the two drainage systems meet.

The larvae of D when alive differ from those of C by having an over-all dark reddish-brown color and darkened head capsule whereas living larvae of C are light brown in color with light head capsules. Collections of sibling D adults reared in 1956 were given to the Entomology Division, Department of Agriculture, Ottawa, and adults of C reared in 1957 were given to D. M. Wood for further study.

Siblings E and F were recognized on the basis of material sent by J. A. Rubtzov from four streams in the vicinity of Leningrad, U.S.S.R. (Table III). No further information on the collecting sites is available.

The larvae of E and F are evidently almost impossible to distinguish. By conventional taxonomic procedure Rubtzov identified larvae from streams No. 1 and No. 2 (Table III) as *Eusimulium securiformes* Rubtzov, which he had previously split from the *E. "aureum"* complex. Among these *E. securiformes*, he recognized two different but closely related forms which he referred to loosely as *E.* aff. *aureum* sp. 1 (stream No. 1) and *E.* aff. *aureum* sp. 2 (stream No. 2). Cytologically these two samples proved to be almost entirely pure for siblings E or F respectively (Table III). The third vial, from stream No. 3, labelled *E. aureum* aff. sp. 3, contained one larvae of E and two of a species with three chromosomes, identified as *Boophthora erythrocephala* D.G., the larvae of which apparently intergrade morphologically with those of

TABLE III

Collection data for siblings E and F (see Table I)

Location	Collection date	Frequency of sibling	
		E	F
Leningrad Area			
Stream No. 1	May 30, 1957	(1)	(10)
Stream No. 2	May 31, 1957	(16)	(1)
Stream No. 3	Aug. 7, 1957	(1)	
Stream No. 4	Aug. 14, 1957	(5)	(11)
Great Britain			
England	Aug. 8, 1958		(13)
Scotland	Aug. 15, 1958		(10)

TABLE IV

Collection data for form G (see Table I)

Location	Collection date	Frequency of form G
California		
Berkeley	Aug. 16, 1955	(12)
Topaz Lake	June 25, 1958	(5)

E. "aureum". Material labelled *E. aureum* aff. sp. 4, from a fourth stream, was a mixture of siblings E and F, and *B. erythrocephala*, and presumably represents later generations of E and F. Collections of *E. "aureum"* made by D. M. Davies from northwest England and northwest Scotland conformed cytologically in all essential respects to sibling F from Leningrad (Table III). Specifically the two collecting sites involved are described thus: (1) small tarn above Easedale Tarn near Grassmere (Lake Windemere district), Westmorland, England, and (2) top of hill (pond outlet) northeast of Loch Maree near Ananeaun Nature Conservatory Station, Kinlocheure Ross-shire, Scotland.

Two samples from California were recognized as representing a seventh form subsequently called G. One sample was collected by William Bentinck from a small stream on the campus of the University of California at Berkeley, California, while the other was obtained by D. M. Wood from a stream running into Topaz Lake on the California-Nevada border (Table IV).

Material fixed at Churchill, Leningrad, or in the western United States and sent by air to Toronto in 3:1 acetic alcohol gave satisfactory results, even though one lot of Russian material was a month in transit. The slides were prepared by the standard techniques of this laboratory (8, 14) except that dry ice was not available at Churchill, where expanding CO_2 gas was used to freeze the preparations.

Results

General Features of the Salivary Gland Chromosomes

The seven forms of *E. "aureum"* dealt with in this paper are so similar in their gross cytological characters that the general descriptions of the mitotic complement and of the salivary gland chromosomes, as given previously (8) for siblings A and B, apply to all. The relative lengths of salivary gland chromosomes as proposed in per cent total complement length for A and B (8) are essentially identical for all forms. Only in form E was the tight pairing occasionally relaxed for indeterminate lengths so that the two constituents could be distinguished easily.

Certain larvae of C when counterstained with fast green showed a secondary nucleolar organizer in section 27B in the expanded region of the first chromosome (Figs. 3, 4). A second nucleolus at this point was also found in some slides of D (Fig. 5) but in no other form.

In one larva of B from Oregon what appeared to be ectopic pairing between the postulated centromeres was noticed in a number of nuclei. Three examples are given to support the previous contention that the expanded region of the long chromosome has two centromeres (C_1 and C_2) while that of the short chromosome has only one (C_3) (Figs. 11–13).

TABLE V

Number of larvae identified (F') and number completely analyzed cytologically (F'') (parentheses denote form totals)

Form	Area	Period	F'	F''
A	Toronto	1952–1956	209	203
	Montreal	1952, 1953	4	4
	Toronto	1957, 1958	11	11
	Gatineau	1958	92	89
			(316)	(307)
B	Toronto	1952–1956	435	435
	Montreal	1952, 1953	7	7
	Toronto	1957, 1958	36	36
	Oregon	1958	3	3
			(481)	(481)
C	Churchill	1951–1957	(163)	(157)
D	Churchill	1951–1956	(208)	(187)
E	Leningrad	1957	(23)	(23)
F	Leningrad	1957	22	22
	England	1958	23	18
			(45)	(40)
G	Berkeley	1955	12	12
	Topaz Lake	1958	5	5
			(17)	(17)
		Total	1,253	1,212

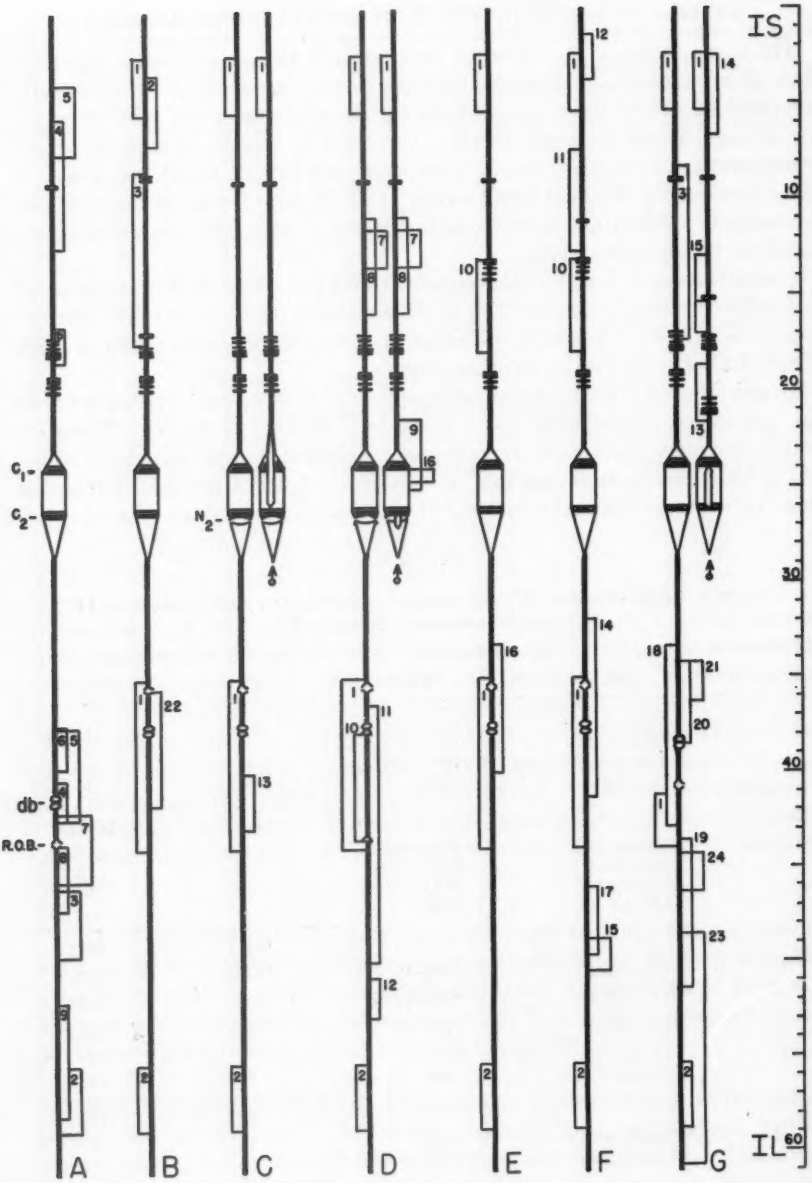


FIG. 1

NOMENCLATURE

In the first paper on siblings A and B (8), for reasons of convenience, A was chosen as standard. The salivary gland chromosome complement of A was then divided into 100 sections and B was described in terms of A. In this paper the banding sequences of all forms are referred directly to A

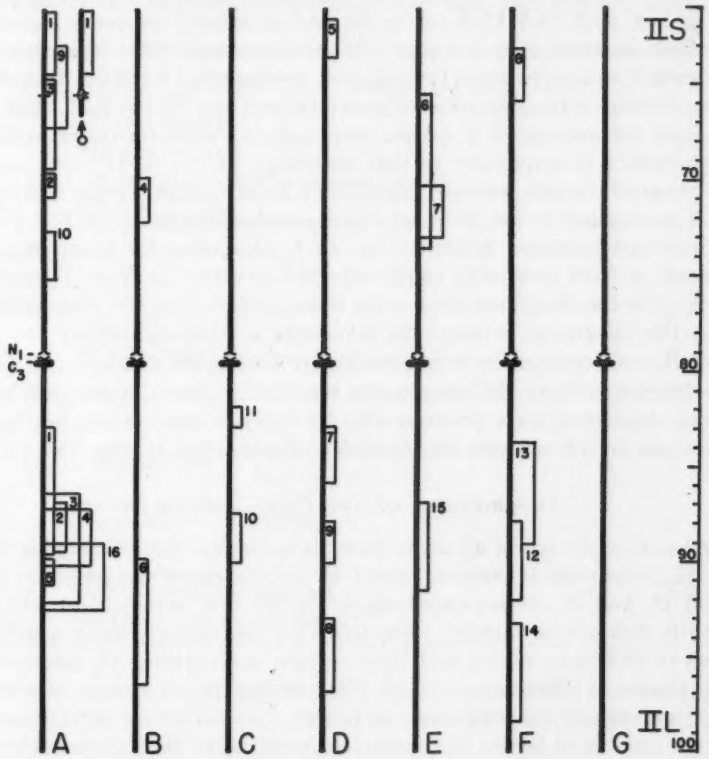


FIG. 1. Salivary gland chromosome idiograms of *E. "aureum"* siblings A to G. C_1, C_2, and C_3 = centromeres (8); N_1 and N_2 = nucleoli; R.o.B. = Ring of Balbiani (14); *db* = "double bubble".

as standard, but this becomes increasingly unwieldly since certain forms differ by many rearrangements from A (Fig. 1). As a result of recent studies the choice of A as standard is rather unfortunate since other forms, like C or Hy, are more central phylogenetically (Fig. 37). In any case, in future studies it may be necessary to use a double standard (1), that is, to renumber consecutively the basic sequence for each form in order to describe the intraspecific inversions, and to describe the interspecific inversions in terms of a universal standard for the species complex. At present in the idiograms the interspecific inversions are represented as shifting the banding sequence of the universal standard (A) to form new standards for each form as indicated

by the change in location of certain markers (Fig. 1). The break points of intraspecific inversions are then plotted against the separate standards of the individual forms. Thus in the idiograms and maps interspecific and intraspecific inversions are referable strictly to individual form standards regardless of complexity.

Inversions in each arm were given consecutive numbers (e.g., 3, 4, 5, etc. in the figures; IS-3, IS-4, IS-5, etc. in the text) in order of discovery regardless of the form in which they occurred. Where inversions differ from standard A by several successive steps (overlapped, overlapping; included, including) the intermediate arrangements are also symbolized (e.g. IS-1.2, IL-1.18.20.21). Sometimes the number of a specific step only is shown for convenience or where reference is specifically to that step (e.g., *IL-18*, IL-21). An exceptional inversion which resulted apparently from a three-break rearrangement is symbolized in the same way as a simple inversion (*IS-15*). In the text, italicized inversion symbols (e.g. *IS-3*, *IL-1.10*) refer to interspecific inversions, or fixed inversions found restricted to either an *X* or *Y* sequence (*IS-13*). On the idiograms (and maps when viewed with the same orientation as the idiograms) interspecific inversions are represented by brackets to the left, and intraspecific inversions by brackets to the right.

The brackets denote the locations of the breakpoints. In the case of an inversion dependent on a previous step its bracket stops at the bracket of the previous inversion above the location of the breakpoint (Fig. 2).

Descriptions of the Complements

The banding pattern in all seven forms is so similar that a complete band for band comparison is possible except for certain minor discrepancies in A (8) and D, and in the expanded region of the first chromosome which is inherently difficult to analyze. The following descriptions entail a detailed analysis of each arm, taking each form in turn and covering (1) interspecific (fixed) inversion differences from A, (2) intraspecific (floating) inversions, and (3) unresolved discrepancies. Inversion frequencies are determined by using the number of larvae completely analyzed rather than the total identified (Table V). The reader is referred to tables VI to IX and XV to XVIII for inversion limits and frequencies.

First Chromosome, Short Arm, IS

Form A.—The basic arrangement of A is adopted as standard for this study (8, Figs. 8, 11) (Fig. 1). The intraspecific inversions IS-4 and IS-5 are found in both Ontario and Quebec while IS-6 is known only from Quebec (Tables VI, XV)

Form B.—Form B, in Ontario and Quebec, differs from A by three fixed inversion steps, *IS-1*, IS-1.2, and *IS-3*. When populations of B from eastern Canada only were available IS-2 was considered an interspecific inversion (8, 9) but the finding of IS-2 only once and heterozygously in three larvae of B from Oregon (Tables VI, XVI) changed its status to intraspecific. A

TABLE VI

Description of the inversions found in the short arm of the first chromosomes of forms A to G

(All inversions are described in terms of the standard A sequence. Arrangements are described by giving the end bands of the rearranged segment or segments along with the end bands of the standard sequences between which the rearrangement occurs, the segments being separated by periods. *F'*, frequency of homozygotes; *F''*, frequency of heterozygotes; % of constituents. Arrangements are described only once, when they first appear in the table)

Arrangement	Description	*F'*	*F''*	%
Standard A	1A1–25C	87		
IS-4	6B2.13B1–6B3.13B2	71	133	44.8
IS-5	4C4.8B1–4C5.8B2		7	1.1
IS-6	17B.19A5–17C1.19B1		2	0.3
Standard B	1A1–3B1.6B2–3B2.6B3–9A4.18B3–9B1.18B4–25C	2		
IS-1	3B1.6B2–3B2.6B3			
IS-3	9A4.18B3–9B1.18B4			
IS-1.2	3B1.6B2–5C1.7C3–6B3.3B2–5B4.8A1	478	1	99.5
Standard C	1A1–3B1.6B2–3B2.6B3–25C	157		
IS-1				
Standard D	As in C	127		
IS-1				
IS-7	12B5.14B2–12B6.14B3		21	5.6
IS-8	11C2.16C2–11C3.16C3		1	0.3
IS-9 ♂	22C4.26A1–22C5.26A2		36	9.6
IS-16 ♂	24C1.25A2–24C2.25B1		4	1.1
Standard E	1A1–3B1.6B2–3B2.6B3–14A2.18C5–14A3.19A1–25C	23		
IS-1				
IS-10	14A2.18C5–14A3.19A1			
Standard F	1A1–3B1.6B2–3B2.6B3–8B1.13C1–8B2.13C2–14A2.18C5–14A3.19A1–25C	36		
IS-1				
IS-10				
IS-11	8B1.13C1–8B2.13C2			
IS-1.12	2B1.5A1.6B2.3B1–2B2.4C7–3B2.6B3		4	5.0
Standard G	As in C	1		
IS-1				
IS-13 ♂	19B7.22B3–19C1.32C1		12	35.3
IS-15 ♂	14A1.15C1–18A2.14A2–15B3.18A3		12	35.3
IS-3 ♀		4	8	47.1
IS-1.14	3B1.6B2–6B1.7B3–6B3.3B2–6A4.7C1		7	20.6

non-pairing section from 25A1 to 28A1 with a non-homology in one constituent from 25B to 27C was also found in one male from Oregon and is discussed later in relation to sex determination.

Form C.—The basic arrangement of C is intermediate between A and B in that it differs from A by having the interspecific inversion, *IS-1*, and from B by not having *IS-3*. There are no known intraspecific inversions (Figs. 1, 17–19). In 47 slides a non-pairing section 22B1 to 26C was regularly observed in males only (Figs. 6, 7; Table XI). This specific failure in pairing is apparently associated with a cytological sex-determining mechanism and is discussed in a later section. Non-pairing in the expanded region was also noted in a few females, but the section was much shorter and not regularly delimited, even within one slide.

Form D.—This form has the same basic arrangement as C. Four intraspecific inversions were found, IS-7, IS-8, IS-9, and IS-16 (Figs. 1, 14–19). IS-9 is the commonest floating inversion (Figs. 15, 16, 18), being found 36 times heterozygously but not once homozygously. Furthermore 30 of these

heterozygous larvae were sexed by gonad dissection and all were males (Table XII). This inversion is further discussed in a later section in relation to sex determination. It is readily identified, being the only inversion in any form to extend into the expanded region. IS-7 occurred 19 times heterozygously both in males and females, and twice associated with IS-9. IS-8 was found only once; on the map it includes IS-7 (Figs. 1, 17, 19). IS-16, the smallest inversion found in any form, involves only three bands and is unique in being located entirely within the expanded region (Figs. 1, 14, 18). It was found only four times heterozygously, each time in a male, which suggests that it too may be restricted to the *Y* chromosome. In 31 specimens a small, deeply staining heterochromatic "inclusion" was found on one constituent only (Fig. 10). Of these individuals 24 were sexed and all were male (Table XIII). This "inclusion" was not found homozygously. These facts suggest that this "inclusion" too is restricted to the *Y* chromosome and is so shown in the idiogram. In one larva it was associated with IS-7.

Form E.—The basic arrangement of E differs from that of C by one additional homozygous inversion, *IS-10*, so that E differs from A by having both *IS-1* and *IS-10* (Figs. 1, 20, 21). No floating inversions were found in this sibling.

Form F.—This form is distinguished from E by an additional simple inversion *IS-11*, so that it differs from A by having *IS-1*, *IS-10*, and *IS-11* (Figs. 1, 20, 21). IS-12 (IS-1.12), the only floating inversion in this arm (Fig. 21), was found only once in the Leningrad sample and in a slide too poor to allow complete analysis, but what is presumed to be the same inversion in the British samples was found heterozygously four times (Table XVII); the limits shown are based on this analysis (Table VI).

Form G.—Determination of the basic sequence in G is somewhat complicated because this arm is involved in cytological sex determination, and the basic sequences of both the *X* and *Y* chromosomes are entirely different. Unfortunately gonadal sexing was not carried out for the sample from Berkeley. The indirect evidence is as follows. Upon analysis of the Berkeley sample only, it was found that some larvae were completely and identically homozygous for the short arm and tightly paired in the expanded region, whereas all other larvae had a complex series of heterozygous inversion configurations in three regions (one in two regions only, Fig. 22) and an unpaired expanded region (Fig. 8). Evidently the short arm exists in the population essentially as two alternative sequences differing by a multiple rearrangement. The absence of intermediate conditions (heterozygotes in one or two sequences only), the absence of homozygotes corresponding to one of the inversion sequences, and the extension of heterozygosity to the expanded region all suggest we are here dealing with a complex chromosomal sex-determining mechanism similar to that already discussed for certain *Prosimulium* (12). On this interpretation the arrangement in the homozygous condition would correspond to the *X* sequence. The *X* sequence is identical with the standard arrangement in B having *IS-1* and IS-3, and no floating inversions were associated with this arrangement. The *Y* arrange-

ment is basically as in C having *IS-1*, but having in addition two fixed inversions *IS-15* and *IS-13*, and in seven out of eight cases the floating inversions IS-14 (IS-1.14) (Table VI). IS-1.14 forms a simple loop with *IS-1* in seven of the eight slides assumed to be male. The second configuration is rather complex, involving IS-3 paired with *IS-15* (Figs. 22, 23). When plotted on standard, *IS-15* is seen to be included in IS-3 (Fig. 1). The third configuration in all eight slides is another simple loop formed by *IS-13* pairing with standard (Figs. 22, 23). In the expanded region of the presumed males there is a well-defined break in the tight pairing from 24C to 27A (Fig. 8) similar to those found in B and C (Figs. 6, 7, 9). Apparently *IS-15* is a rare example of a three-break rearrangement in which the two sections involved have changed places but maintained their original orientation. The sequence could be represented as 1.4–5.2–3.6 illustrating the three-break rearrangement indicated by Rothfels (15) as not having been found before. (It is possible that *IS-15* is the result of three tandem inversions, and if this is so a more intensive survey of the range of *E. "aureum"* might turn up the intermediate steps.) The complex heterozygous configuration in Berkeley males is illustrated in Fig. 23. In summary the *X* arrangement from Berkeley has *IS-1* and IS-3, while the *Y* arrangements appear to have *IS-1*, *IS-13* plus *IS-15*, and the preceding three plus IS-1.14. The four males and one female from the Topaz Lake sample sexed by examining the gonads strongly support the above interpretation, for the four males are homozygous for *IS-1*, are heterozygous for *IS-13* and *IS-15* (but lack IS-1.14, Table VI), and are unpaired in the same part of the expanded region, while the female is homozygous for *IS-1* and has the tightly paired expanded region. It should be noted that both the *X* and *Y* sequence from Topaz Lake lack IS-3. Apparently then IS-3 is a floating inversion restricted to the females of form G, but it also functions as an interspecific inversion in form B (8). Finally, considering both samples of G, *IS-1* is fixed in both the *X* and *Y* sequences, *IS-13* and *IS-15* are fixed in the *Y* sequence, IS-3 is floating in the *X* sequence, and IS-1.14 is floating in the *Y* sequence.

First Chromosome, Long Arm, IL

Form A.—The basic arrangement of A was chosen as standard for this arm (8, Figs. 8, 15) (Fig. 1). Seven intraspecific inversions have been described: IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, and IL-2.9 (8) (Table VII). In the over-all sample, IL-3 was found in 45% of the constituents (Table VII). But when the frequency of this inversion was examined regionally it was found in southern Ontario in 33%, and in the Gatineau Hills populations in 73% of the constituents (Table XV). Only two other inversions, IL-4 and IL-5, were observed in both populations from southern Ontario and Quebec (Table XV).

Form B.—Form B differs from A by having *IL-1* and *IL-2*. This same arrangement is also basic for forms C, E, and F (8, Figs. 8, 14, 16) (Figs. 1, 24, 27). No floating inversions were found in the eastern populations, but one, IL-22 (IL-1.22) (Table VII), was found in one western larva in association with IS-2.

TABLE VII

Descriptions of the inversions found in the long arm of the first chromosome of forms A to G (see Table VI)

Arrangement	Description	*F'*	*F''*	%
Standard A	26A1–61C	66		
IL-3	47A.50B4–47B1.50C1	60	158	45.3
IL-4	41A.43A2–41B1.43A3		52	8.5
IL-5	38B.43A2–38C1.43A3		10	1.6
IL-6	38B1.40B3–38B2.40C1		3	0.5
IL-7	42C.46C4–43A1.47A1		2	0.3
IL-8	44B.48A2–44C1.48A3		1	0.2
IL-2.9	52C.59A1–59C2.56A4–53A1.58C5–56B1.59C3		2	0.3
Standard B	26A1–35C.44C5–36A1.45A1–56A4.49C2–56B1.59C3–61C	480		
IL-1	35C.44C5–36A1.45A1			
IL-2	56A4.49C2–56B1.59C3			
IL-1.22	35C.44C5–44B(–1).38B1–44B(–2).38A6		1	0.1
Standard C	As in B	156		
IL-1				
IL-2				
IL-1.13	35C.44C5–40A1.37A1–39C.36C–36A1.45A1		1	0.3
Standard D	26A1–35C.44C5–42A2.36B2–42A1.36B1–36A1.45A1–56A4.49C2–56B1.59C3–61C	8		
IL-1.10	35C.44C5–42A2.36B2–42A1.36B1–36A1.45A1			
IL-2				
IL-1.10.11	35C.44C5–43C1.50C4–45A1.36A1–36B1.42A1–36B2.42A2–43B5.51A1	99	78	73.8
IL-12	51C4.53C4–51C5.53C5	2	16	5.4
Standard E	As in B	21		
IL-1				
IL-2				
IL-1.16	34A4.40A1–44C5.35C–34A5.39C–36A1.45A1		2	4.4
Standard F	As in B	12		
IL-1				
IL-2				
IL-1.14	32C3.38C1–44C5.35C–32C4.38B3–36A1.45A1	18	9	56.2
IL-15	49C1.51B1–49C2.51B2		4	5.0
IL-17	47A1.50B3–47A2.50B4		1	1.3
Standard G	26A1–34A5.37A3–44C5.35C–34B1.37A2–36A1.45A1–61C	0		
IL-1.18	34A5.37A3–44C5.35C–34B1.37A2–36A1.45A1			
IL-2		1	8	29.4
IL-1.18.19	36B3.52B1–45A1.36A1–36B2.52B2	12	3	79.4
IL-1.18.20	37C6.42A3–38A1.42B1	14	3	91.2
IL-1.18.20.21	37C6.39C3–42A4.39C2		4	11.8
IL-23	49B3.61C3.49B4.61C4		5	14.7
IL-24	45A6.47A3–45B1.47B1		1	2.9

Form C.—Basically C is like B for this arm (Figs. 24–27). One intraspecific inversion, IL-1.13 (Figs. 1, 24, 27), turned up once. A secondary nucleolar organizer in section 27B was found in many slides (Figs. 1, 3, 4). Without fast green counterstaining it was very difficult to be sure of its presence in poorer preparations. It was definitely not present in all slides and occasionally it took over for the main nucleolus (in the short chromosome), which was missing.

Form D.—Form D differs from B by one step, the addition of an included inversion *IL-10* (*IL-1.10*) (Figs. 28, 29). There are two floating inversions in this arm, IL-11 (IL-1.10.11) and IL-12 (Figs. 1, 28, 29). The largest intraspecific inversion so far found in any form, IL-1.10.11, involves three-eighths of the arm. It forms a large, simple loop when heterozygous with the standard arrangement for D. It is very common, being found in 74% of the constituents (Table VII). IL-12 was found 14 times heterozygously and twice homozygously, both alone and associated with IL-1.10.11. Two

minor discrepancies were found in this arm. The more common one, found in both sexes, was an unpaired region from 45A to 46B. This was confined to about 80% of the heterozygotes between IL-1.10.11 and *IL-1.10* with one exception, when it was noted in a slide homozygous for *IL-1.10*. Another discrepancy in segment 27B in the expanded region (Figs. 15, 16) was found 11 times, each time in association with IS-9, thus apparently associated with sex determination. The secondary nucleolus of the long chromosome, in section 27B (Figs. 1, 5), was also found in this sibling, although not as frequently as in C. In a few larvae at least it also took over from the main nucleolus of the short chromosome. The secondary nucleolus was never found in association with the discrepancy mentioned above, although the two are located in the same section.

Form E.—This form has the same basic arrangement as B. One intraspecific inversion was noted twice heterozygously IL-16 (IL-1.16) (Table VII; Figs. 1, 30).

Form F.—The arrangement in F is again as in B. Three intraspecific inversions are known, IL-14 (IL-1.14), IL-15, and IL-17 (Figs. 1, 30). IL-1.14 was found 8 times heterozygously and once homozygously and standard 13 times homozygously in the Russian samples while in the British material, 17 larvae (out of 18 analyzed) were homozygous, and one heterozygous, for IL-1.14 (Figs. 1, 30; Tables VII, XVII). The remaining two intraspecific inversions are known only from the Russian material (Tables VII, XVII). IL-15 was found four times heterozygously and always in association with IL-1.14. IL-17 was found once heterozygously. IL-15 and IL-17 overlap on the map (Figs. 1, 30). One interesting point about IL-17 is the remarkable closeness of its breakpoints to those of IL-3 (Table VII).

Form G.—This form is related to B by a single large inversion, *IL-18* (*IL-1.18*), which overlaps *IL-1* distally and reinverts more than 85% of *IL-1* (Figs. 1, 2, 31–33). Six intraspecific inversions IL-2, IL-19, IL-20, IL-21, IL-23, and IL-24 (Table VII) are found in this form usually in somewhat involved inversion sequences. IL-19 (IL-1.19), like *IL-1.18*, overlaps *IL-1* distally leaving only a small portion of *IL-1* unchanged (Figs. 1, 2, 31–33). IL-20 (IL-1.18.20) is in that section of *IL-1* reinverted by *IL-18* and presumably evolved in the sequence shown in Fig. 2. IL-21 (IL-1.18-.20.21) is in turn included in IL-1.18.20 and inverts a small section of standard A for a fourth time. These last two inversions, IL-20 and IL-21 (Figs. 1, 32, 33), have one breakpoint in common and are independent of the other four floating inversions found in this arm of form G. In the Berkeley sample IL-19 and IL-20 are found homozygously in all 12 larvae giving rise to the complex and apparently fixed inversion sequence IL-1.18.19.20 (Figs. 2, 31–33) and in one of these larvae IL-21 was found heterozygously giving the sequence IL-1.18.19.20.21 (Tables VII, XVIII). In the Topaz Lake sample of five larvae IL-19, IL-20, and IL-21 were each found three times heterozygously and IL-20 homozygously twice. Thus the inversions IL-19 and IL-20, which appeared interspecific from the Berkeley sample, are actually intraspecific (Fig. 1). It is noteworthy that for the thesis study (9) for which

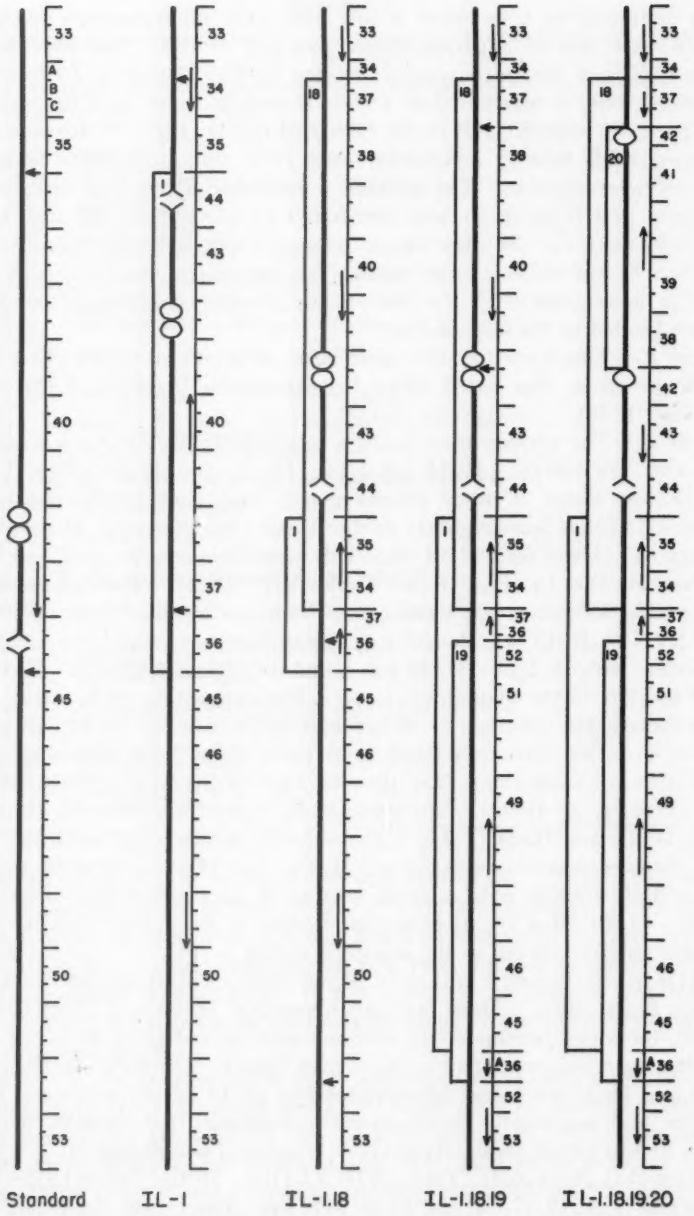


FIG. 2. Stepwise development of the complex inversion sequence IL-1.18.19.20 in G. Horizontal arrows in one sequence indicate the breakpoints of the next inversion step. Vertical arrows indicate the orientation of the segments.

the Berkeley sample only was available, the hypothetical intermediate steps, IL-19 and IL-20, were worked out. These were later verified by the Topaz Lake larvae. The next two inversions, IL-23 and IL-24, are from the Topaz Lake sample only (Table XVIII). IL-23 is a large subterminal inversion which on the map overlaps IL-19 and includes IL-2 (Figs. 1, 31). It was found heterozygously in all five larvae. IL-24 is a small independent inversion included in IL-1.19 on the map (Figs. 1, 33). It was found only once associated with IL-1.18.20 and IL-24 forming three simple heterozygous loops. IL-2 was found seven times heterozygously and once homozygously in the Berkeley sample but only once heterozygously in the Topaz Lake sample (Figs. 31, 33). It should be pointed out that IL-2, which is a floating inversion in G, functions as an interspecific inversion between A and the other five forms, B to F (Fig. 1). Because of the various associations between the six floating inversions in this arm in the Topaz Lake larvae, there were two or three, often complex, heterozygous configurations.

Second Chromosome, Short Arm, IIS

No interspecific differences were found in either arm of the short chromosome. Consequently the arrangements here are confined to intraspecific inversions in each form (Tables VIII, IX; Fig. 1) (8, Figs. 17–25).

Form A.—Five inversions, IIS-1, IIS-2, IIS-3 (8), IIS-9 (IIS-1.9), and IIS-10 (Table VIII), were found. In the Quebec populations IIS-3 was not found, while the proportions of IIS-1 and IIS-2 (Table XV) remain essentially unchanged from the Toronto populations. The two new rare inversions, IIS-1.9 and IIS-10, were found heterozygously in the Quebec larvae (Tables VIII, XV).

TABLE VIII

Description of the inversions found in the short arm of the second chromosome of forms A to G (see Table VI)

Arrangement	Description	*F'*	*F''*	%
Standard A	62A1–80A	1		
IIS-1	62A2.65B2–62A3.65B3	261	45	92.4
IIS-2	65B5.70A4–65C1.70B1		30	4.9
IIS-3	70B2.71C2–70B3.71C3		1	0.2
IIS-1.9	62A2.65B2–63C4.68A1–65B2.62A3–63C3.68A2		1	0.2
IIS-10	73C3.76A1–74A1.76A2		2	0.3
Standard B	As in A	476		
IIS-4	70C4.73A2–70C5.73A3		5	0.5
Standard C	As in A	157		
Standard D	As in A	186		
IIS-5	62B4.64B5–62B5.64B6		1	0.3
Standard E	As in A	14		
IIS-6	66B1.74B3–66B2.74B4		4	8.7
IIS-7	71A4.73C3–71A5.74A1		5	10.9
Standard F	As in A	28		
IIS-8	64A1.66C2–64A2.66C3		12	15.0
Standard G	As in A	17		

Form B.—One inversion IIS-4 (8) was found and only in the Toronto area (Table VIII).

Form C.—No inversions were found.

Form D.—One inversion, IIS-5, was found once heterozygously (Table VIII).

Form E.—Two inversions were found heterozygously. IIS-6 was found four times and is included on the map in IIS-7, which occurred five times. The two were not recorded together (Table VIII).

Form F.—One inversion, IIS-8, was found heterozygously only, three times in the Leningrad sample and eight times in the British sample (Tables VIII, XVII).

Form G.—No inversions were found.

Second Chromosome, Long Arm, IIL

Form A.—Six inversions were found in this form: IIL-1, IIL-1.2, IIL-3, IIL-4, IIL-5 (8), and IIL-16 (Table IX, Fig. 1). In the Quebec populations the proportions of IIL-1, IIL-1.2, IIL-3, and IIL-4 are essentially the same as in Ontario while IIL-16 was found only once (Table XV); IIL-5 is not known in Quebec.

Form B.—One rare inversion, IIL-6, was found (8) and only in the Toronto area (Tables IX, XVI).

Form C.—Two rare independent inversions were recorded, IIL-10 twice and IIL-11 three times (Table IX). Both were found associated twice, and IIL-11 by itself once.

FIGS. 3, 5. Forms C and D; secondary nucleolus in 27B. FIG. 4. Form C; fast green preparation showing ectopic pairing between the two nucleoli in 27B and 80A. FIGS. 6, 7. Form C; limits of the unpaired section in the expanded region of males. FIGS. 8, 9. Forms G and B; unpaired section in the expanded region of males. FIG. 10. Form D; "inclusion" in 12B. FIGS. 11–13. Form B; ectopic pairing between centromeres (C_1 and C_2; C_2 and C_3; C_1 and C_3). FIG. 14. Form D; standard/IS-16. FIGS. 15, 16. Form D; standard/IS-9, and unpaired section in 27B. FIGS. 17–21. Long chromosome, short arm, IS. The floating inversions of form D, whose standard band sequence is not shown, are illustrated on form C and distinguished with subscripts. FIGS. 17–19. Form C; standard band sequence. FIGS. 20, 21. Form F; standard band sequence. FIG. 22. Form G; male sequence showing heterozygous configurations for IS-3/*IS-15* and standard/*IS-13*. Subscripts on figure numbers refer to the forms.

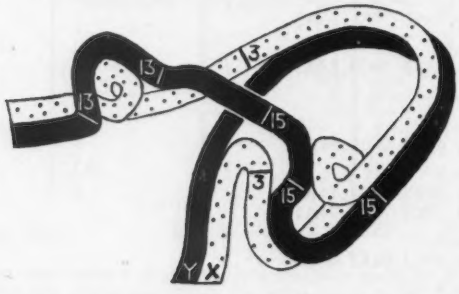
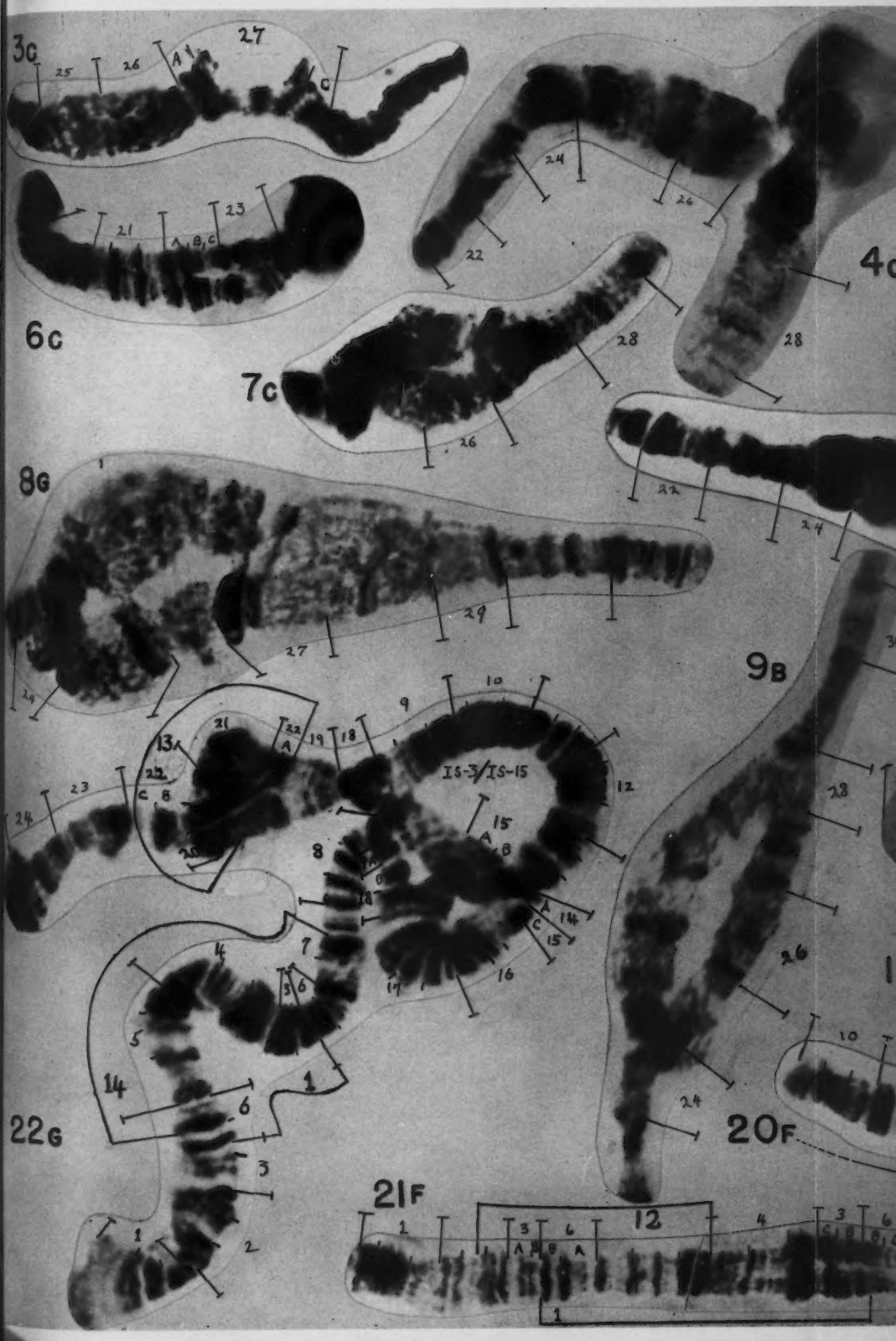
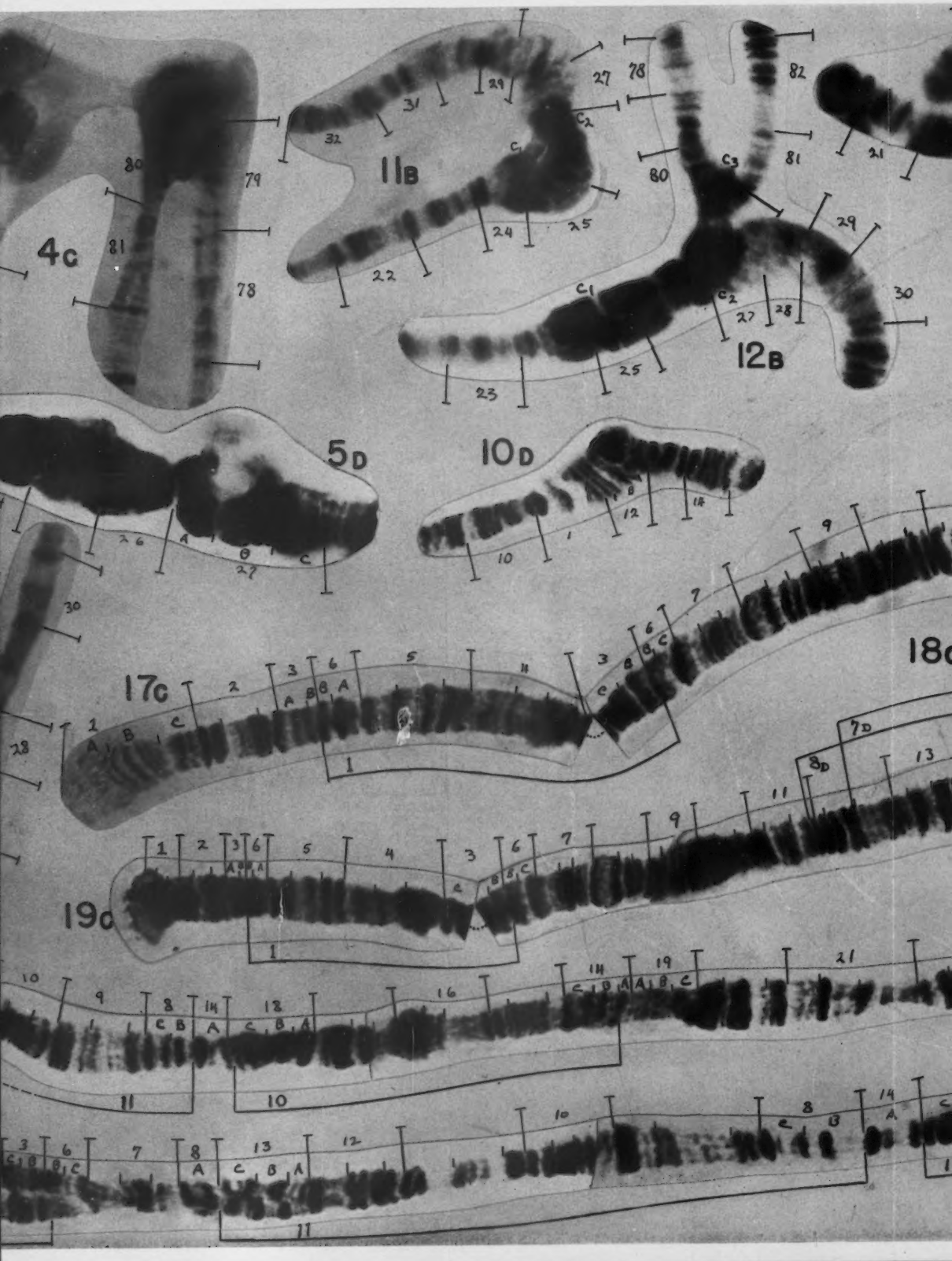
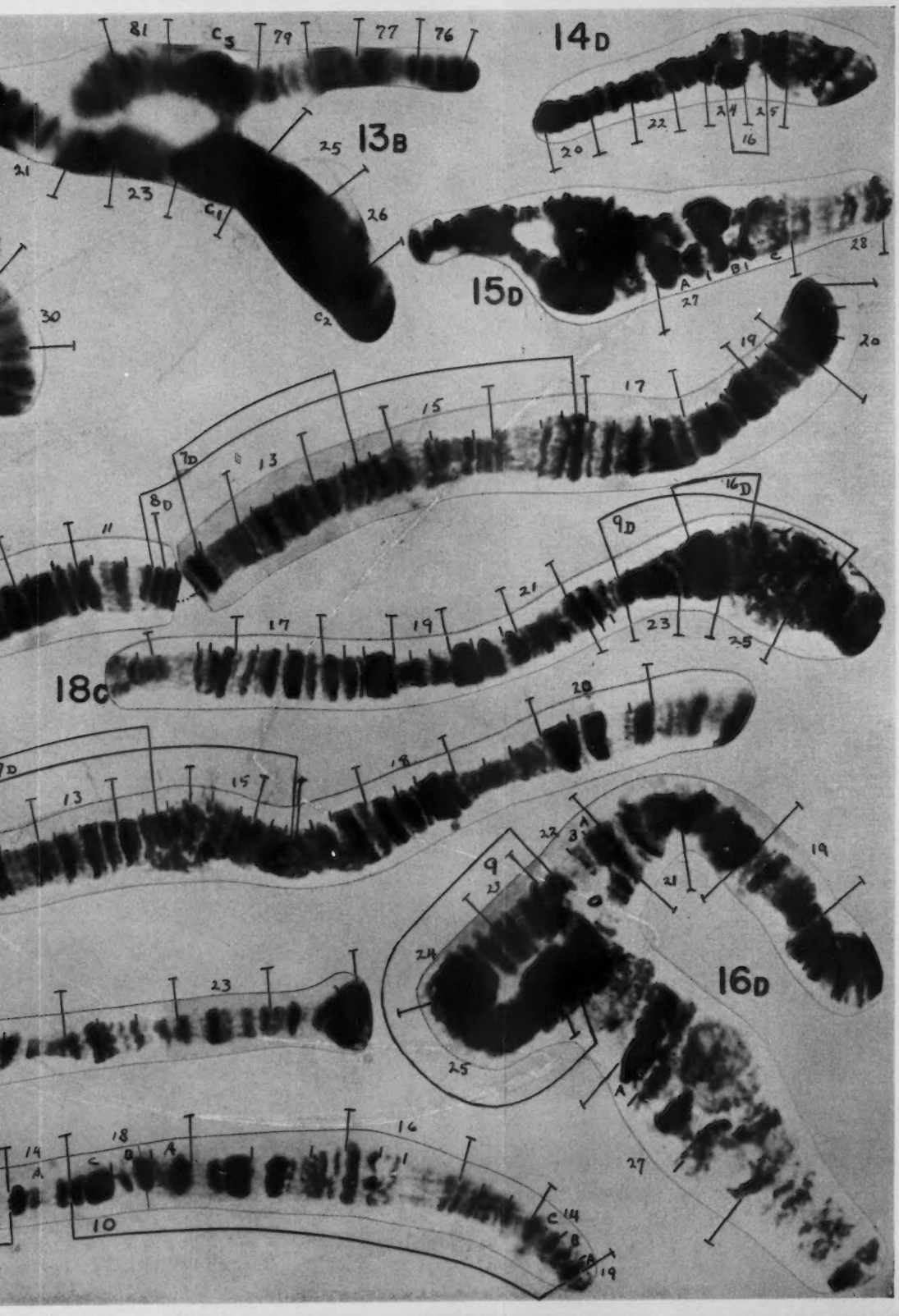


FIG. 23. Diagram illustrating the pairing between the *X* and *Y* sequences in Fig. 22.


3C
6C
7C
4C
8G
9B
22G
20F
21F
IS-3/IS-15


4c
11B
12B
5D
10D
17c
18c
19c
7D
8D


81
C5
79
77
76
13B
25
21
23
C1
26
C2
30
14D
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24
25
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28
A
B1
C
27
19
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7D
13
8D
11
16D
9D
21
23
25
18C
17
19
20
18
15
13
23
24
25
16D
27
14
18
A
B
C
10
19

TABLE IX

Descriptions of the inversions found in the long arm of the second chromosome of forms A to G (see Table VI)

Arrangement	Description	F'	F''	%
Standard A	80B1–100C	136		
IIL-1	83C4.90B5–83C5.90B6	12	108	21.5
IIL-1.2	83C4.90B5–90B4.87C2–90B3·87C1–83C5·90B6		16	2.6
IIL-3	88A2.91A2–88A3.91A3		28	4.6
IIL-4	87B1.93A2–87B2.93A3		6	1.0
IIL-5	90C1.92A1–90C2.92A2		1	0.2
IIL-16	89C4.93B1–90A1.93B2		1	0.2
Standard B	As in A	479		
IIL-6	90C1.97B1–90C2.97B2		2	0.2
Standard C	As in A	154		
IIL-10	88A.80C–88B1.91A1		2	0.6
IIL-11	82B.83C3–82C1.83C4		3	0.9
Standard D	As in A	182		
IIL-7	83C3.86C2–83C4.86C3		2	0.5
IIL-8	83C4.97C1–93C5.97C2		2	0.5
IIL-9	88C1.90C–88C2.91A1		1	0.3
Standard E	As in A	21		
IIL-15	87C1.92B1–87C2.92B2		2	4.4
Standard F	As in A	0		
IIL-12	88C2.92C3–88C3.93A1	14	13	52.1
IIL-12.13	84B4.90A1–92C3.88C1–84C1.89C4–88C2.93A1	10	14	42.5
IIL-14	94A2.99A3–94A3.99B1		1	1.3
Standard G	As in A	17		

Form D.—Three rare independent inversions were recorded for this form. IIL-7 and IIL-8 were each found twice and IIL-9 was found only once (Table IX).

Form E.—One inversion IIL-15 was found twice heterozygously (Table IX).

Form F.—Three inversions were recorded for this form: IIL-12, IIL-13 (IIL-12.13), and IIL-14 (Fig. 1). For the Leningrad sample IIL-12 was found only once paired with standard and nine times with IIL-12.13 producing simple loops (Tables IX, XVII). IIL-12.13 was also found paired with standard twice, giving an overlapping configuration, and 10 times homozygously. These were the only combinations found in the samples from Leningrad. Of the British specimens 14 were homozygous for IIL-12, two were heterozygous for IIL-12/IIL-12.13, one was heterozygous for standard/IIL-12, and one other was heterozygous for standard/IIL-12.13. The frequency of two of the elements in this triad (17, 18), IIL-12 and IIL-12.13, between the British and Russian material is essentially reversed, while standard remains uncommon (Table XVII). The independent subterminal inversion IIL-14 was found only once heterozygously and in the Russian material where it was associated with a simple loop formed by IIL-12/IIL-12.13.

Form G.—There were no inversions found.

Sex-Determining Mechanisms

Sexing of the larvae was carried out by squashing the immature gonads after they were stained along with the salivary glands. It was found in A, C, D, and G definitely, and in B possibly, that the males were frequently or always heterozygous for certain arrangements or showed non-pairing in the expanded region. With the exception of the extra bands in A, these characters are confined to the short arm and expanded region of the long chromosome. The females on the other hand invariably were homozygous for only one of the alternate sequences and tightly paired in the expanded region. The most readily accepted interpretation of these facts assumes that certain arrangements are restricted to genetic *X* and *Y* chromosomes respectively. Sex determination is of the *XX* or *XY* type with males being the heterogenetic sex. The details are given below.

Form A.—In section 66B (Fig. 34) an extra heavy band in one constituent only and associated with a short non-pairing segment was found in 31 larvae from the Toronto area. Twenty-five of these larvae had been sexed and all were male (Table X). Similarly in the Quebec samples 12 males and no females were found with this extra band (Table X). Three females from

TABLE X

The relation between the extra band in 66B and sex in form A

	S/S	S/E*	E/E
♂ Ontario	17	25	0
Quebec	17	12	0
♀ Ontario	54	0	0
Quebec	27	0	0

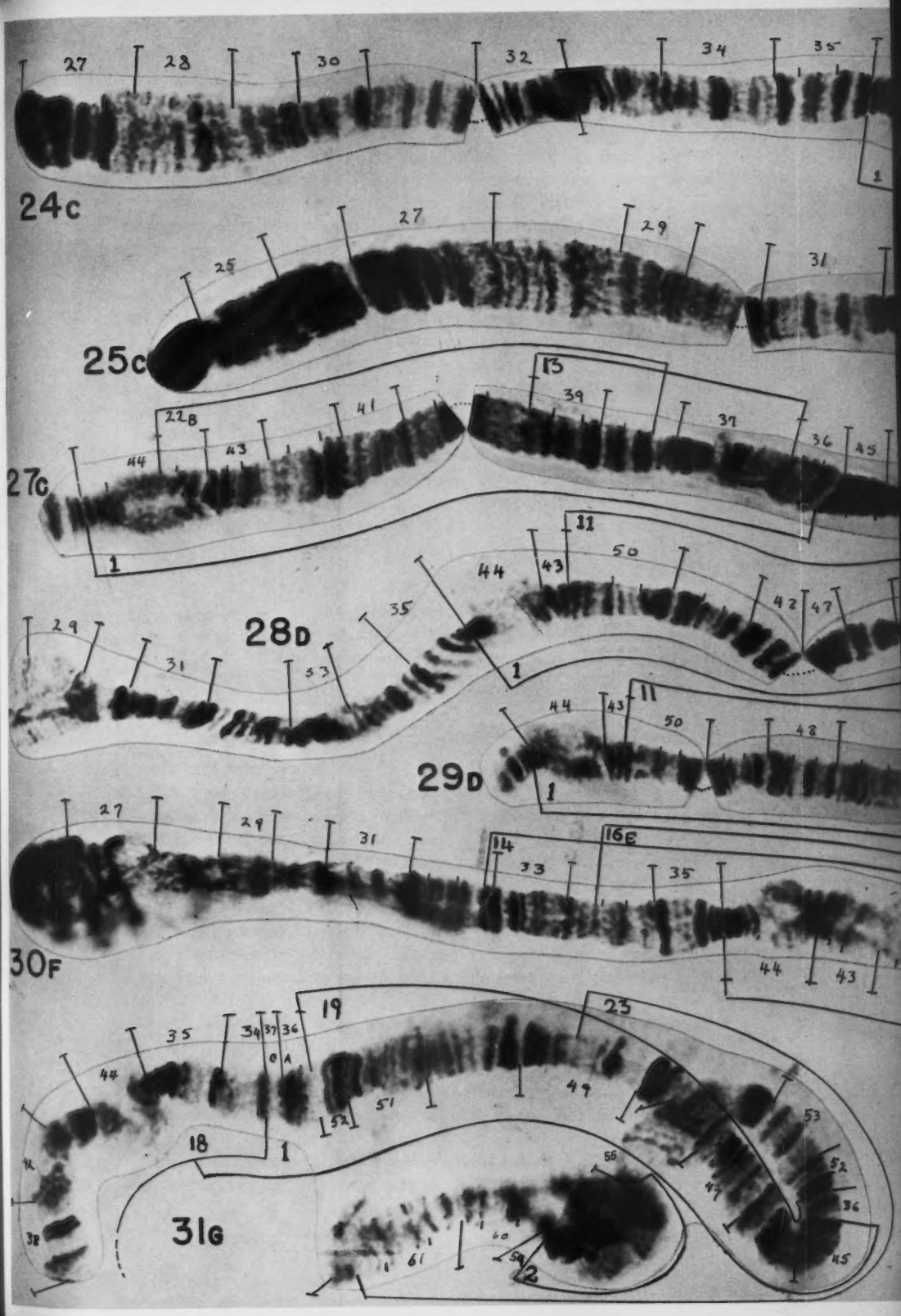
*E = Extra band.

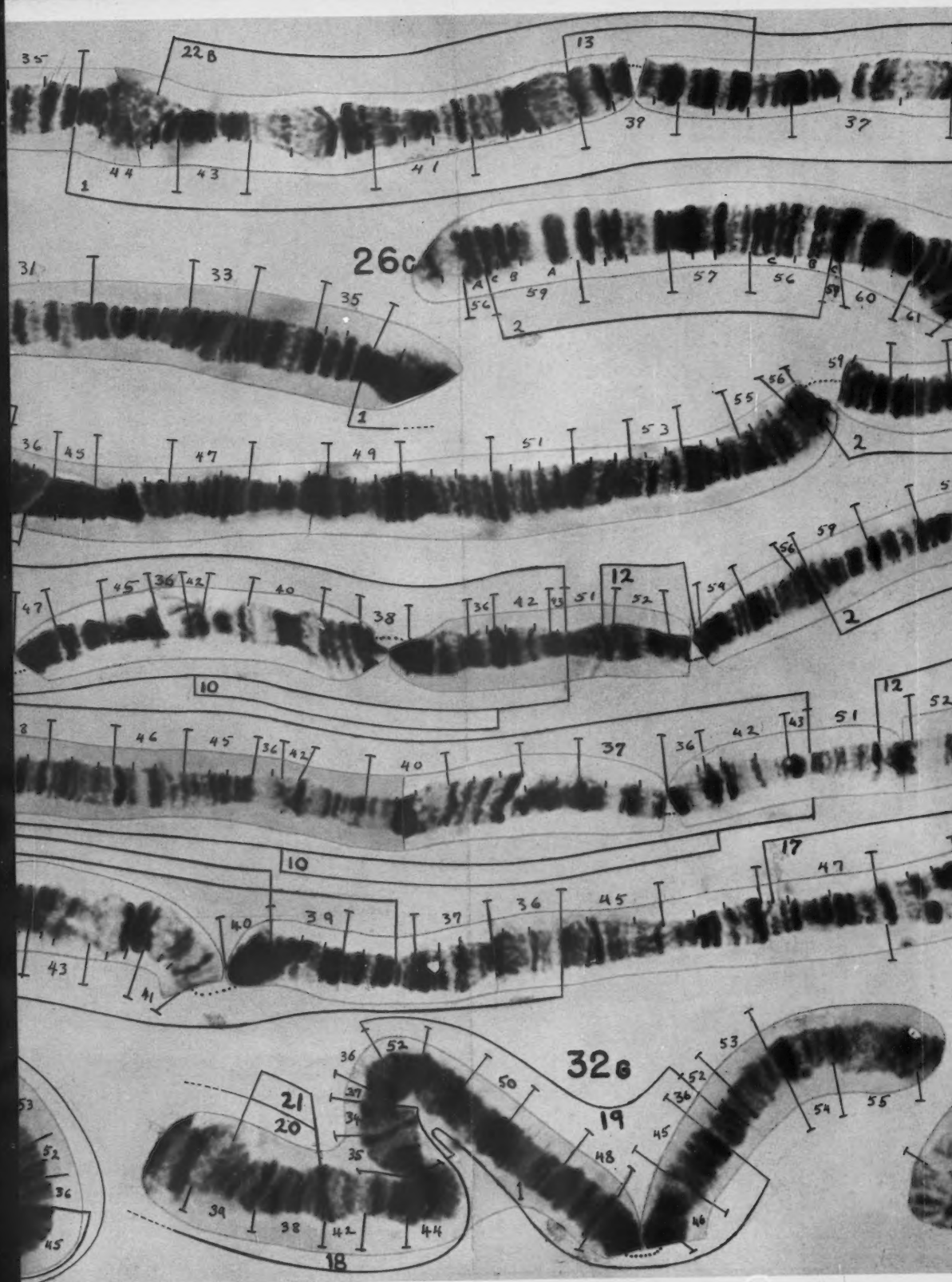
Ontario and two from Quebec were found which did not pair in 66B but they did not have the extra band found in one constituent in some males. At present no interpretation can be given for the failure to pair in 66B in certain females. The normal condition is illustrated in Figs. 35 and 36. It may be concluded then that in A there are two different *Y* chromosomes, one indistinguishable from the *X* chromosome having an extra, heavy band in 66B as well as the ability to cause non-pairing in this section.

Form B.—The evidence for a sex-determining mechanism in B is only regional and very meager. Of the three larvae from Oregon two were males and one of these showed non-pairing in the expanded region from 25A1 to

Figs. 24–33. Long chromosome, long arm IL. The floating inversions of forms B and E, whose standard band sequences are not shown, are illustrated on forms C and F, and distinguished with subscripts. Figs. 24–27. Form C; standard band sequence. Figs. 28, 29. Form D; band sequence homozygous for IL-1.10.11. Fig. 30. Form F; standard band sequence.

Figs. 31–33. Form G; band sequence homozygous for IL-1.18.19.20 and heterozygous for IL-2. Fig. 34. Form A; 66B, heterozygous for the extra, heavy band of the *Y* sequence and the associated unpaired section. Figs. 35, 36. Forms A and B; 66B, homozygous for standard. Subscripts on figure numbers refer to the forms.


24c
25c
27c
28D
29D
30F
31G


35
22B
13
39
37
1
44
43
41
26C
31
33
35
A C B A
59
57
56
60
61
2
1
36
45
47
49
51
53
55
56
59
2
47
45
36
42
40
38
36
42
43
51
52
12
59
56
59
2
10
8
46
45
36
42
40
37
36
42
43
51
12
52
10
17
47
43
41
40
39
37
36
45
32G
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54
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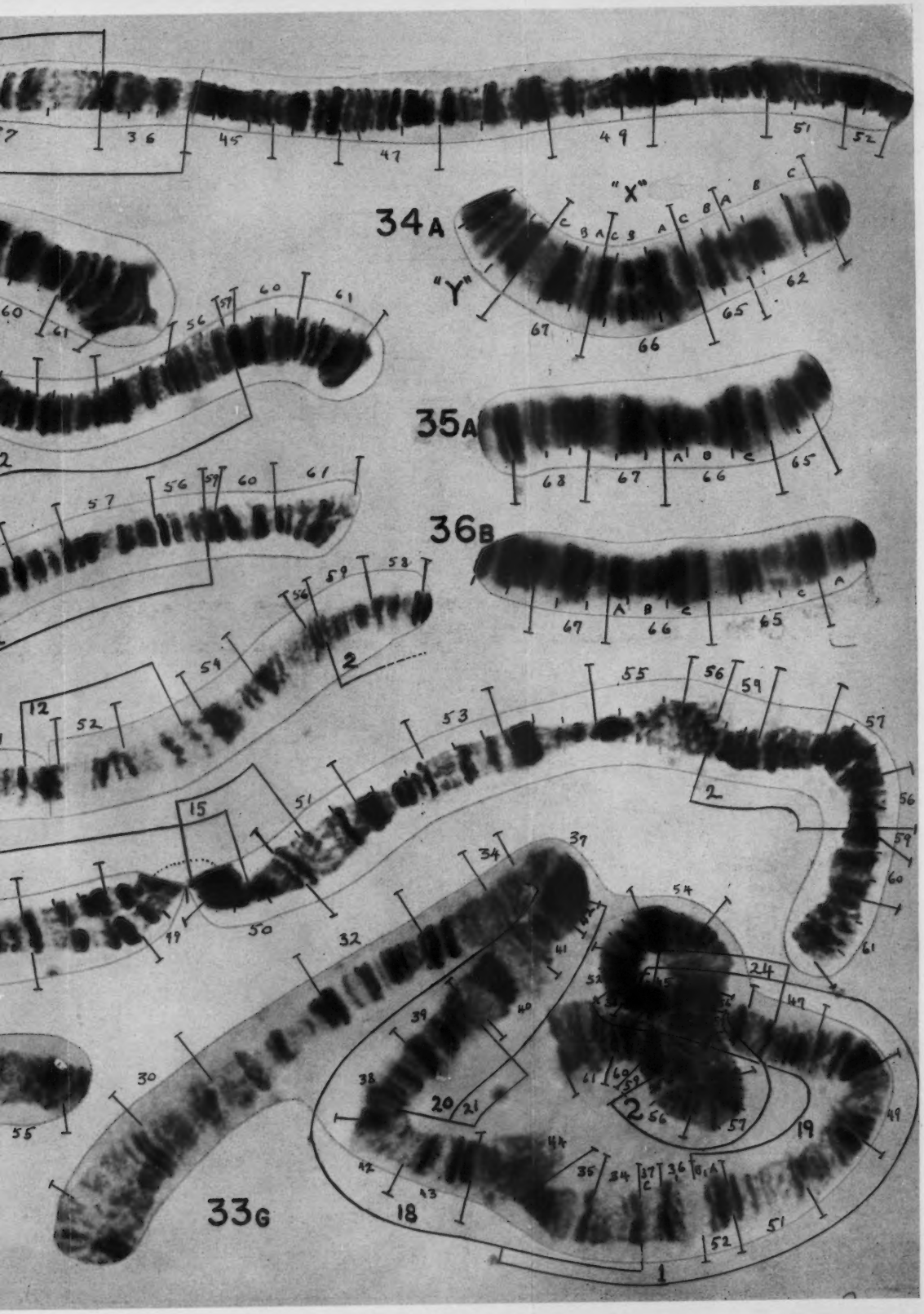
39
38
42
44
46


34A
"X"
"Y"
35A
36B
33G

28A1 (Fig. 9). Also included in the non-pairing part of the expanded region is a non-homologous section from 25B to 27C which may or may not be an inversion. This non-pairing resembles quite closely the conditions described below for the expanded regions of C or G (Figs. 6–8), and the position of the non-homology resembles that of IS-16 in D (Fig. 14). Because of the remarkable similarity between the expression of the expanded region in this single larva of B and characteristics of the expanded region in C, D, and G, the author considers that at least western populations of B probably have distinct *X* and *Y* chromosomes. No distinguishing features for *X* and *Y* chromosomes are known for eastern populations of B.

Form C.—The tight pairing so characteristic of the species was relaxed in 62 larvae from 22B1 to 26C (Figs. 6, 7) so that the two homologues spread apart. Forty-six out of fifty of the gonad-sexed males and none of the females showed this lack of pairing (Table XI), clearly reflecting a disparity between the *X* chromosome and certain *Y* chromosomes. The sex of the four exceptional males was confirmed on re-examimation, indicating the existence in the population of two different *Y* chromosomes, those that induce a lack of pairing in the expanded region, and those that do not.

TABLE XI

The relation between non-pairing (from 22B1 to 26C) and sex in form C

	Paired	Non-paired
♂	4	46
♀	44	0

Form D.—In this form several cytological features appear to be restricted to males. The most noticeable is the inversion IS-9 (Figs. 1, 15, 16, 18) which includes part of the expanded region. This occurred in a third of all males (Table XII). A second common feature of *Y* chromosomes is the "inclusion" between 12B3 and 4 (Fig. 10) which characterizes approximately one-quarter of the known males (Table XIII). Oddly enough, it was associated with IS-9 only once. Thirdly, a small unpaired region in section 27B (Figs. 15, 16) was observed 11 times, each time in association with IS-9, so it too must be characteristic of certain *Y* chromosomes. Finally, the inversion IS-16 was found only four times, each time in a male. On the maps and idiograms (Figs. 1, 14, 18) IS-16 is included in IS-9 but the two were never found together. Since in this same region in males of B, C, and G there is specific non-pairing, and in D there is the male restricted inversion IS-9, IS-16 is also considered to be restricted to males.

Forms E and F.—No evidence of any form of chromosomal heterozygosity associated with sex-determining mechanisms was found in either of these two forms.

Form G.—Of all the forms in which there is evidence of a cytological sex determining mechanism, G is the only one in which the *Y* chromosome can

TABLE XII

The relation between the inversion IS-9 and sex in form D

	S/S	S/9	9/9
♂	55	31	0
♀	45	0	0

TABLE XIII

The relationship between the "inclusion" (between 12B3 and 4) and sex in form D

	S/S	S/I*	I/I
♂	62	24	0
♀	45	0	0

*I = Inclusion.

TABLE XIV

The relationship between the inversions IS-3, *IS-13*, IS-1.14, and *IS-15*, and non-pairing; and sex in form G

	$\frac{S(+3)}{S(+3)}$ +P*	$\frac{S(+3)}{13+15(+14)}$ +NP*	$\frac{13+15(+14)}{13+15(+14)}$
Berkeley, presumed (♂)	0	8	0
Topaz Lake (♂)	0	4	0
Berkeley, presumed (♀)	4	0	0
Topaz Lake (♀)	1	0	0

*P = paired, NP = non-paired.

always be distinguished from the *X* chromosome. The *Y* chromosome underwent a series of changes which resulted in two sequences, one having *IS-1*, *IS-13*, IS-1.14, *IS-15*, and the inability to pair in the expanded region, the other like the first but lacking IS-1.14 (Figs. 1, 22, 23). Similarly two different *X* chromosomes are known, the basic one with only *IS-1*, and the derived one with IS-3 in addition. IS-3 was never found on *Y* chromosomes.

The seven forms of *E. "aureum"* can be placed in four categories according to the cytology of sex determination. (1) In E, F, and most of B, there are no distinguishing features between the *X* and *Y* chromosome. (2) In C, and the western sample of B, there were no visible distinguishing marks but in nearly all males there was in the expanded region of the long chromosome a non-pairing segment with definite limits. (3) In A, certain *Y* chromosomes differ from the *X* chromosome by the insertion of at least an extra, heavy band. (4) Finally, in most males of D, and all of G, several distinct *Y* chromosome types are recognized, depending on the occurrence of various rearrangements, non-pairing segments, or discrepancies. A cytologically undifferentiated *Y* chromosome still occurs in D, but has been

eliminated in G. In addition, in G two alternate types of *X* chromosomes are found. Sex-determining mechanisms analogous to 1, 3, and 4 above have also been found in *Prosimulium* (13) and *Chironomus* (6).

Since forms B (in Ontario), E, and F have no sex-determining mechanism the ancestral form, as well, from which the *E. "aureum"* complex arose probably had no sex-determining mechanism or at best had a non-pairing segment in the centromere region of the first chromosome optionally in the male.

Cytological Characterization of All Seven Forms

The basic arrangement of form A was chosen as the standard in terms of which all other sequences are expressed (8). Known interspecific differences in the other six forms are confined strictly to the long chromosome, the shorter chromosome having the same basic sequence in all forms. Intraspecific inversions may be found throughout both chromosomes. Tables VI to IX and XV to XVIII are referred to for all inversions, their limits, and their frequencies.

Form A.—Populations of A have a total of 21 floating inversions, making this the most polymorphic of all the forms. The populations in southern Ontario have 17 of these floating inversions, four of which, one in each arm, are very common: IS-4 (50% of the constituents), IL-3 (33%), IIS-1 (91%), and IIL-1 (22%). Two others are rather uncommon in their occurrence, IL-4 (8%)

TABLE XV

Frequency of inversions in populations of form A from Ontario and Quebec

(n = the total number of constituents examined; f = the number of constituents for the given inversion)

Inversion	Ontario ($n = 428$)		Quebec ($n = 186$)	
	f	%	f	%
IS-4	213	49.8	62	33.3
IS-5	4	0.9	3	1.6
IS-6			2	1.1
IL-3	143	33.4	135	72.6
IL-4	35	8.2	20	10.8
IL-5	8	1.9	1	0.5
IL-6	3	0.7		
IL-7	2	0.5		
IL-8	1	0.2		
IL-2.9	2	0.5		
IIS-1	391	91.3	177	95.2
IIS-2	25	5.8	5	2.7
IIS-3	1	0.2		
IIS-1.9			1	0.5
IIS-10			2	1.1
IIL-1	92	21.5	40	21.5
IIL-1.2	10	2.3	6	3.2
IIL-3	18	4.2	10	5.4
IIL-4	5	1.2	1	0.5
IIL-5	1	0.2		
IIL-16			1	0.5

and IIS-2 (6%). The rest are considered rare being found in from 4% to less than 1% of the constituents. All 11 of the inversions from southern Ontario found in 1% or more of the constituents (Table XV) are also found in southwestern Quebec. The same four inversions as before were common, IS-4 (33%), IL-3 (73%), IIS-1 (95%), and IIL-1 (22%), but the proportions of two of these have changed considerably. Four new and rare inversions were found making a total of 15 from Quebec samples. In both Ontario and Quebec populations extra bands in the shorter chromosome are evidently restricted to certain *Y* sequences.

Form B.—Form B differs from A by four inversion steps, *IS-1* and *IS-3* in the short arm, and *IL-1* and *IL-2* in the long arm. In eastern populations the floating inversion IS-1.2 is found in 100% of the constituents where it is essentially a fifth homozygous inversion difference between A and B (Table XVI). Two other floating inversions, IIS-4 and IIL-6, which are rare are known in the east, both being found in less than 1% of the constituents.

TABLE XVI

Frequency of inversions in populations of form B from Ontario and Oregon (see Table XV)

	Ontario (n = 962)		Oregon (n = 6)	
Inversion	f	%	f	%
IS-2	962	100.0	1	(16)
IL-22			1	(16)
IIS-4	5	0.5		
IIL-6	2	0.2		

The small western sample of three larvae had two homozygous for standard B. The third had IS-1.2 heterozygously as well as the fourth floating inversion known for B, IL-1.22 (Table XVI). A non-pairing segment with an included discrepancy in the expanded region of the long chromosome in this last larva resembled similar segments in C and G and was taken as the only possible method of distinguishing the *X* and *Y* chromosomes known for form B. Because of the general homogeneity of the chromosomes (only 6 larvae out of 478 with even one floating inversion), polymorphism in B, at least in eastern populations, appears to be suppressed.

Form C.—This form differs from A by having three homozygous inversions, *IS-1*, *IL-1*, and *IL-2*, and from B by not having *IS-3*. Intraspecific inversions are rare, only three, IL-1.13, IIL-10, and IIL-11, being known. These were found in 4 (out of 163) larvae; polymorphism again, as in B, appears to be suppressed. A secondary nucleolar organizer was located in section 27B. It was not present in all slides and occasionally replaced the nucleolus in the short chromosome. A large, non-pairing section from 22B1 to 26C in most males was presumed to be caused by subtle differences between the *X* and chromosomes which could not be determined from the banding pattern.

Form D.—Form D differs from A by having one more interspecific step than C, an inversion included in *IL-1*, *IL-1.10*. Ten intraspecific inversions are known, making this another polymorphic species. One of these, IL-11 (IL-1.10.11), was very common, being found in 74% of the constituents. IS-7 (6%), IS-9 (10%), and IL-12 (5%) were rather uncommon. The rest were rare, IS-8, IIS-5, IIL-7, IIL-8, and IIL-9 being found once or twice and IS-16 four times. Standard D was found homozygously throughout the complement only three times. IS-9, in roughly the same position as the split in the first chromosome of the males of C, is apparently associated with sex determination. In addition, three other characters, (1) an inclusion between 12B3 and 4, (2) IS-16, and (3) a short unpaired section in section 27B, are found exclusively in certain males. The secondary nucleolus of the first chromosome was also found in this form, although not as frequently as in C. In three slides it had replaced the nucleolus of the second chromosome.

Form E.—Form E differs from A by four homozygous inversions. It is like C in having *IS-1*, *IL-1*, and *IL-2*, but in addition E also has *IS-10*. Four intraspecific inversions were noted, IL-1.16, IIS-6, IIS-7, and IIL-15, none of which were very common. About half the slides had heterozygous inversions, the other half were homozygous for standard E. No sex-determining mechanism was found.

Form F.—Form F differs from E by the simple interspecific inversion *IS-11*, and has as well *IS-1*, *IS-10*, *IL-1*, and *IL-2*. In the Leningrad sample eight intraspecific inversions are known in F, four more than in E. Three of these are common, IL-1.14 (23% of the constituents), IIL-12 (23%), and IIL-12.13 (70%). The other five are uncommon or rare, IS-1.12 (2%), IL-15, IL-17, IIS-8 (9%), and IIL-14. The two samples of F from England and Scotland agree with F from Leningrad. No new floating inversions were discovered but five out of eight of the inversions found in larvae from Leningrad were also found in larvae from Britain, IL-1.14 (97%), IIl-12 (86%), IIL-12.13 (8%), IS-1.12 (11%), and IIS-8 (22%), although with changed proportions in every case (Table XVII). Particularly, in England, the standard sequence alternate to IL-1.14 has almost disappeared, and in

TABLE XVII

Frequency of inversions in populations of form F from British and Russian samples (see Table XV)

Inversion	Leningrad ($n=44$)		Britain ($n=36$)	
	f	%	*f*	%
IS-1.12	1	2.3	4	11.1
IL-1.14	10	22.8	35	97.3
IL-15	4	9.1		
IL-17	1	2.3		
IIS-8	4	9.1	8	22.2
Standard (alternate to IIL-12)	3	6.8	2	5.6
IIL-12	10	22.8	31	86.2
IIL-12.13	31	70.5	3	8.3
IIL-14	1	2.3		

the triad in the long arm of the second chromosome standard remains uncommon whereas IIL-12 and IIL-12.13 have reversed proportions from the Leningrad sample. The frequent occurrence of floating inversions makes this another polymorphic form. No sex-determining mechanism was found.

Form G.—This form differs from A in the short arm of the long chromosome in both the *X* and *Y* sequences by *IS-1*, and in the *Y* sequence by *IS-13* and *IS-15* as well. In the long arm both sexes differ from A by the overlapping inversion *IL-18* (*IL-1.18*). Polymorphism in this form is confined to the long chromosome, since no inversions were found in the short chromosome. In the Berkeley sample the two floating inversions in the short arm, IS-1.14 found heterozygously in seven out of eight males, and IS-3, found homozygously in all females and heterozygously in all males, are apparently restricted to the *Y* and *X* sequences respectively. In the long arm IL-21 was found only once and heterozygously, and IL-2 was found seven times heterozygously and once homozygously. It should be noted that both IL-19 and IL-20 were postulated as the hypothetical intermediates necessary for the derivation of the Berkeley sequence, IL-1.18.19.20 (9), which was provisionally thought to be the standard sequence for G (Fig. 2). The analysis of the Topaz Lake sample proves this interpretation correct as both IL-19 and IL-20 were discovered heterozygously (Table XVIII). IL-21 and IL-2 were found heterozygously along with two new inversion heterozygotes, IL-23 and IL-24. In both samples there is a non-pairing section from 24C to 27A, in the same part of the expanded region as the non-pairing sections in B and C, and also associated with the *XY* condition.

TABLE XVIII

Frequency of inversions in populations of form G from Berkeley and Topaz Lake samples (see Table XV)

Inversion	Berkeley ($n=24$)		Topaz Lake ($n=10$)	
	f	%	*f*	%
IS-3	20	83		
IS-13	8	33	4	(40)
IS-1.14	7	29		
IS-15	8	33	4	(40)
IL-2	9	38	1	(10)
IL-1.18.19	24	100	3	(30)
IL-1.18.20	24	100	4	(40)
IL-1.18.20.21	1	4	3	(30)
IL-23			5	(50)
IL-24			1	(10)

Discussion

Phylogenetic Relationships between All Seven Forms

The idiograms (Fig. 1) are graphic representations of the gross features of the polytene complements of all seven forms, showing in addition the interspecific and intraspecific inversions including the differential segments

of the *X* and *Y* chromosomes. In order to elucidate phylogenetic relationships, which are not shown directly by the idiograms, the principles of overlapping inversions and shared arrangements (11) may be used. The most useful arrangements are those that are shared by several forms and these are restricted entirely to the long chromosome. When the inversions limited to the short arm are considered, one possible scheme of relationships is obtained; when the inversions of the long arm only are used, a somewhat different scheme results. The linear series D, C, E, and F is common to both schemes and offers no problems but conflicting indications are obtained regarding the relations between A, B, C, and G in which IS-3 and IL-2 occur in various combinations. In order to reconcile these differences and combine these two schemes into one it is necessary either to assume that the same arrangement arose independently more than once, or to postulate a central hypothetical intermediary (Hy) in which both IS-3 and IL-2 were floating. Since it is highly improbable that the same inversion would arise independently twice (7) the second method was followed in drawing up the chart in Fig. 37.

Starting with A (Fig. 37) the first evolutionary step is then the derivation of Hy by the acquisition of two fixed inversions, *IS-1* and *IL-1*, and one floating inversion, IS-3, and by IL-2 remaining floating (IL-2 is assumed floating in A as the yet-undiscovered intermediary between standard and IL-2.9).

From Hy there are three easily derived independent lines which lead to forms B, C, and G. Form B is the stabilized form of Hy in which *IS-3* and *IL-2* are fixed, while C is the stabilized form of Hy in which *IL-2* and the standard sequence alternative to IS-3 are fixed; but neither can be Hy as

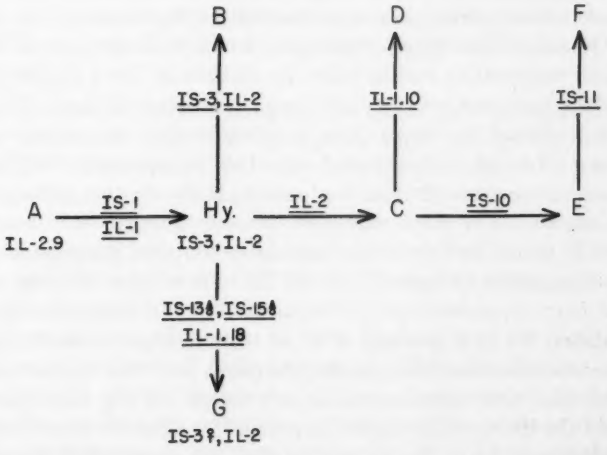


FIG. 37. Phylogenetic chart of seven forms of *E. "aureum"*. Inversions marked along the arrows become fixed in that step for the following forms. Inversions listed below the letters are floating in that form.

they lack the necessary alternative sequences to derive other forms. G is also close to Hy in that it has IS-3 (in the *X* chromosome only) and IL-2 as floating arrangements but differs by an additional fixed inversion, *IL-18* (*IL-1.18*), and by two fixed inversions restricted to the *Y* chromosome. From C, forms D and E are derived by the fixation of single inversion steps, D by the addition of *IL-10* which is included in *IL-1*, and E by the addition of the independent inversion *IS-10*. Finally F is derived from E by the addition of one further simple independent inversion *IS-11*. The choice of form A as the standard of reference was dictated only by considerations of convenience in the original descriptions of forms A and B (8). Although this usage has been adhered to in the present study, it is not implied that A is necessarily the ancestral form. A more logical choice would be a more central form such as the polymorphic hypothetical Hy with four known lines of development, or possibly C with three such lines.

Taxonomic Status of the Seven Forms

Sympatric Sibling Species Pairs

Siblings A and B

Larvae from southern Ontario were found to segregate into two quite distinct cytological groups (8) referred to as siblings A and B. In this area the band sequence of B differs from that of A as the result of five inversion steps, *IS-1*, IS-1.2, *IS-3*, *IL-1*, and *IL-2* (8, Fig. 1). The shorter chromosome showed no such differences. Superimposed on these two basic arrangements were, in A, 17 floating inversions scattered throughout both chromosomes, and some of them quite common, and in B, only two rare inversions in the short chromosome. The 92 larvae from the Gatineau Hills north of Ottawa were entirely form A and conformed to the basic pattern for A in southern Ontario. The Quebec samples, which include larvae from Prefontaine and Montreal as well as from the Gatineaus, have 15 floating inversions, of which four rare ones are not found in southern Ontario (Table XV). Nine of the 11 shared inversions occur in approximately the same proportions; the other two, IS-4 and IL-3, show significant shifts in proportions (Table XV).

The three larvae from Oregon conformed closely to the pattern for B in southern Ontario. The major difference between Oregon and Ontario larvae is that IS-1.2, found entirely homozygously in Ontario, was found only once and heterozygously in Oregon (Table XVI). Three other floating inversions were found heterozygously, two in Ontario and one in Oregon larvae.

In 797 slides, 316 of A and 481 of B, no instances were recorded of heterozygosity for the so-called interspecific inversions nor were any floating inversions found that were common to the two forms. These facts demonstrate that, within the limits of the rather large sample, effective interbreeding does not occur between A and B. Although they are sympatric at least for part of their ranges, A and B are reproductively isolated and biologically discrete sibling species.

Siblings C and D

Collections from Churchill followed a pattern similar to those in southern Ontario. Again two distinct cytological forms were found differing in the basic sequence in the long chromosome only and each with a distinctive spectrum of floating inversions. Form D differs from C by one interspecific inversion *IL-10*. Superimposed in C are four rare floating inversions; while D has 10 completely different inversions, one of which is very common. Sex determination in C is characterized by non-pairing in the expanded region of the long chromosome of certain males. In D the inversion IS-9 is limited to the *Y* chromosome of certain males, as are the rare inversion IS-16, the "inclusion" between 12B3 and 4, and an unresolved change in section 27B. C and D have one unique character which distinguishes them from other forms so far examined, a second nucleolus in section 27. In 371 slides, 163 of C and 208 of D, the critical heterozygous loop between standard C (*IL-1*) and standard D (*IL-1.10*) was not found, nor were any floating inversions or *Y* chromosome sequences common to the two forms. This lack of swamping of cytological identity again proves the reproductive isolation of the two forms, and establishes the conclusion that C and D are distinct sympatric sibling species.

It may be argued that a limited number of sites provided all the material of these two siblings, and that in fact the two siblings were not found in the same stream. Indeed D was collected only at the inland site and its larvae matured earlier than those of C in the two coastal streams (Table II). However, the difference in the time of appearance of mature larvae may be due essentially to the climatological effect of the frozen bay which delays the opening of the immediate coastal streams by 2 to 3 weeks. The similarity and proximity (10 miles) of the principal collecting sites suggest that the two forms may occur together in the intervening area of flat transitional land which in any case could hardly constitute an ecological barrier.

Siblings E and F

A similar pattern of paired sibling species must again be inferred from the collections taken in the vicinity of Leningrad, where two cytologically distinct forms, E and F, were recognized. In these samples the two forms, like C and D, differ by a single interspecific inversion, *IS-11*, found in sibling F. Superimposed in E are four floating inversions, none of which are common, while F has eight entirely different floating inversions, three of which are quite common. Altogether only 45 slides of these two siblings, 23 of E and 22 of F, were available from the vicinity of Leningrad.

Two further samples of larvae, 13 from England and 10 from Scotland, conformed cytologically to F from Leningrad. Only five floating inversions were found in the British material and these corresponded to five of the eight present in the Russian collections; the proportions of three of the inversions are quite different in the two regions (Table XVII). In these small samples the critical heterozygous loop between standard E and standard F (*IS-11*) was not found nor were any floating inversions common to both

siblings. Since the two forms around Leningrad evidently occur in the same stream for several generations per year (Table III), E and F must be considered as another pair of sympatric sibling species.

Allopatric Forms

All the available material of *E. "aureum"* can be placed in one of seven discrete categories representing the seven forms on the basis of distinctive banding sequences, floating inversions, and sex-determining mechanisms. In the preceding section it was shown on such cytological grounds that the members of the sympatric pairs do not belong to the same biological species. Although the allopatric forms are absolutely distinct in their cytological characteristics, it is conceivable that certain of them are not distinct species but merely geographic variants of a widely distributed polymorphic species. Shifts in the proportion of alternate sequences within such a species might lead to regionally diverse kinds of homozygotes and thus create a semblance of fixed inversion differences between geographically separated populations. The critical heterozygotes may occur in the intervening areas. Any forms tending to such geographically diverse populations might in fact readily interbreed when placed in proximity.

There is evidence in black flies for dissimilar distributions of inversions between various populations of one species. For instance in two populations of *Prosimulium fuscum* Syme and Davies (16) separated by approximately 50 miles there has been complete replacement of 100% standard with 100% of an alternate sequence and reversals in proportions between standard and other floating inversions (13). Mrs. Basrur reports (1) that in two populations of *P. hirtipes* (Fries), one from England and one from Scotland (approximately 180 miles apart), there are no known shared floating inversions, although certain floating inversions are quite common in each of the populations. Similarly in all four forms of *E. "aureum"* for which samples from widely separated localities are available, the frequencies of some floating inversions shift between the different localities. Certain inversions of B (IS-1.2) and of G (IL-1.18.19.20) which are floating in one population occur in 100% of the constituents in another population, while strong shifts in the proportion of floating sequences are found between different populations of A (IS-4, IL-3) and F (IL-1.14, IIL-12, IIL-12.13) (Tables XV, XVII). On the other hand there is evidence for the wide distribution of certain floating inversions. Basrur has reported (2) a complex floating inversion common to natural populations in southern Ontario and Germany of the midge *Glytotendipes barbipes* (Staeger). Similarly in *E. "aureum"*, between populations of form A in southern Ontario and the Gatineaus (about 300 miles apart), there are 11 shared inversions (Table XV). More strikingly, populations of form F from England and Scotland share five floating inversions in roughly the same proportions and these same five inversions, although in somewhat altered proportions, are also found in populations from the vicinity of Leningrad (Table XVII). From the discovery of the existence of such widely distributed inversions it could be expected that at least certain floating

inversions would be common to two or more forms of *E. "aureum"* should they be geographic variants of the same species. The failure to find a single example of such a shared inversion indicates in all probability that the seven forms of *E. "aureum"* are distinct species. Even should a shared inversion be found it may still be in two species as in the case reported by Beerman for the midges *Chironomus tentans* Fabr. and *C. pallidivittatus* Malloch (5).

On phylogenetic grounds among the seven allopatric forms of *E. "aureum"*, at least two can be definitely inferred to be distinct species and a reasonable case can be made for two others. In Fig. 37, D ⟵ C ⟶ E ⟶ F can be considered a lineal series. Since it has been shown that C and D do not interbreed it could hardly be expected that D could cross with either E or F to which it is related directly through C. By the same argument F cannot interbreed with C or D. Similarly both D and F are isolated from any of A, B, or G. Thus the allopatric forms D and F are distinct species.

In discussing phylogeny it was shown that B essentially is one of the homozygous forms of Hy. If the possibility is disregarded that B may have acquired reproductive isolation from A with the loss of the alternate sequences of Hy then it may be concluded that A is another distinct species.

Although only meager samples from the west are on hand, the expectation is that cytological forms like B from central Oregon and G from northern California will probably be found sympatric in the intervening area. Granting this assumption, at least one of B or G is a distinct species, leaving B, C, and E, or G, C, and E as possibly interbreeding yet widely separated geographic forms of a polymorphic species.

Two other and more speculative considerations may be brought to bear upon this problem of allopatric forms. If the phylogenetic sequence illustrated in Fig. 37 is correct, then (considering Hy ancestral rather than A) it is not expected that the polymorphic Hy will be found, it probably having disappeared with the establishment of its various alternate sequences in reproductively isolated forms such as A, B, C, and G. It would thus seem that all four forms, A, B, C, and G, must be distinct species leaving only C and E in a controversial status.

Finally, the differences between the allopatric forms C and E, a single fixed inversion and different sets of floating inversions, are exactly of the kind which distinguish the sympatric species in the pairs C and D, and E and F. Thus in all probability C and E are also reproductively isolated forms. In contrast to other groups, such as fruit flies (11) or midges (4), speciation in black flies (1, 3, 8, 12, 13, 19) appears to be remarkably conservative and in *E. "aureum"* the fixation of a single inversion is evidently capable of producing a new species.

These two main arguments, the one from the failure to find shared floating inversions and the argument from phylogenetic considerations, both lead to the same conclusion, that all seven forms of *E. "aureum"* are

in fact reproductively isolated and can be described as sibling species. This conclusion is admittedly speculative but can be tested by analysis of yet other populations which would indicate the range of each form, and by attempting to cross the forms experimentally.

This leaves the problem of the identification of the black fly described by Fries as *E. aureum* (10) from Lapland. The fact that form F occurs both in upland British streams and streams near Leningrad suggests that it may be the predominant form throughout northern Europe, and thus may be *E. aureum* Fries. However, the possibility that form E from Leningrad also extends into Lapland is not excluded.

Summary

All the larval material on hand purported to be *E. "aureum"*, from North America and Europe, was subjected to an analysis of the giant salivary gland chromosomes. Altogether seven distinct cytological forms were found: three sympatric sibling species pairs, one each from southern Ontario, northern Manitoba, and European Russia; and one solitary form from California.

Distinctions between the forms were made on the basis of basic banding patterns, floating inversions, and the chromosomal sex-determining mechanisms.

The differences between the forms are illustrated by photographic maps of giant chromosomes and by the appropriate idiograms.

Two of the seven forms are clearly distinct. The possibility is not ruled out that some or all of the remaining forms represent cytologically distinct geographic variants of a widely distributed polymorphic species. However, evidence is presented which renders it probable that in fact all seven forms are distinct species.

Acknowledgments

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References

1. BASRUR, P. K. The salivary gland chromosomes of seven segregates of *Prosimulium* (Diptera: Simuliidae) with transformed centromere. Can. J. Zool. **37**, 527–570 (1959).
2. BASRUR, V. R. Inversion polymorphism in the midge *Glyptotendipes barbipes* (Staeger). Chromosoma, **8**, 597–608 (1957).

3. BASRUR, V. R. and ROTHFELS, K. H. Triploidy in natural population of the black fly *Cnephia mutata* (Malloch). Can. J. Zool. **37**, 571–589 (1959).
4. BAUER, H. and BEERMANN, W. Die Polytanie die Riesenchromosomen. Chromosoma, **4**, 630–648 (1952).
5. BEERMANN, W. Chromosomenpolymorphismus und Bastardierung zweier *Chironomus*-Arten. Verhandel. Dtsch. Zool. Ges. Freiburg 1952. Zool. Anz. Suppl. **17**, 290–295 (1953).
6. BEERMANN, W. Geschlechtsbestimmung und Evolution der genetischen Y-Chromosomen bei *Chironomus*. Biol. Zentr. 74, 525–544 (1955).
7. DOBZHANSKY, T. and EPLING, C. Contributions to the genetics, taxonomy and ecology of *Drosophila pseudoobscura* and its relatives. Carnegie Inst. Wash. Publ. No. 554, 1–183 (1944).
8. DUNBAR, R. W. The salivary gland chromosomes of two sibling species of black flies included in *Eusimulium aureum* Fries. Can. J. Zool. **36**, 23–44 (1958).
9. DUNBAR, R. W. The salivary gland chromosomes of seven forms of black flies included in *Eusimulium aureum* Fries. M. A. Thesis, University of Toronto, Toronto, Ontario. 1958.
10. FRIES, B. F. Monographa Simul. Suec. 1824.
11. PATTERSON, J. T. and STONE, W. S. Evolution in the genus *Drosophila*. The Macmillan Co., New York. 1952.
12. ROTHFELS, K. H. Black flies: siblings, sex, and species grouping. J. Heredity, **47**, 113–122 (1956).
13. ROTHFELS, K. H. Unpublished data (1958).
14. ROTHFELS, K. H. and DUNBAR, R. W. The salivary gland chromosomes of the black fly *Simulium vittatum* Zett. Can. J. Zool. **31**, 226–241 (1953).
15. ROTHFELS, K. H. and Fairlie, T. W. The non-random distribution of inversion breaks in the midge *Tendipes decorus*. Can. J. Zool. **35**, 221–263 (1957).
16. SYME, P. D. and DAVIES, D. M. Three new Ontario black flies of the genus *Prosimulium* (Diptera, Simuliidae). Part I; description, morphological comparison with related species, and distribution. Can. Entomologist, **90**, 697–719 (1958).
17. WALLACE, B. Coadaptation and the gene arrangements of *Drosophila pseudoobscura*. I.U.B.S. symposium on Genetics of Population Structure, 67–94 (1953).
18. WALLACE, B. On coadaptation in *Drosophila*. Am. Naturalist, **87**, 343–358 (1953).
19. ZIMRING, R. A comparative study of the salivary gland chromosomes of five related black fly species. M.A. Thesis, University of Toronto, Toronto, Ontario. 1953.

THE SALIVARY GLAND CHROMOSOMES OF SEVEN SEGREGATES OF PROSIMULIUM (DIPTERA: SIMULIIDAE) WITH A TRANSFORMED CENTROMERE[1]

PARVATHI K. BASRUR

Abstract

Preliminary surveys of species of the genus *Prosimulium* led to the discovery of a group of seven forms characterized by a transformation of the centromere of chromosome I. Samples of six of these forms were received from various parts of Europe and the remaining form is native to Ontario. A detailed cytological analysis was conducted on all seven forms with a view to determining their relationship with each other and with the species whose salivary gland chromosomes carry the standard gene arrangement for the genus *Prosimulium*.

In the band comparisons, it was found that all these forms could be distinguished on the basis of characteristic cytological traits such as fixed rearrangements, floating inversions, and in some cases, specific chromosomal sex-determining mechanisms.

Taking the shared, fixed rearrangements as indications of common ancestry, a phylogenetic chart was constructed in which these seven forms were placed in proper relation to one another:

P. fontanum standing alone close to the hypothetical ancestor, *P. hirtipes*, and *P. vigintiquaterni* forming one line of descent, and *P. inflatum* and its allies (*P. inflatum* aff. 1, *P. inflatum* aff. 2, and *P. inflatum* aff. 3) forming another.

Introduction

Species belonging to the genus *Prosimulium* have been subject to one of the several research projects undertaken by this laboratory. During the course of our survey of many *Prosimulium* species from North America and Europe we were impressed by a striking cytological trait common to several species: the presence of a "transformed centromere" region in chromosome I. Preliminary observations proved that all *Prosimulium* species from Europe, except *P. ursinum* Edwards (26), had this characteristic centromere region while all except one of the North American species had what we call the "standard" centromere region. All forms with the transformed centromere exhibit essentially identical morphology in their expanded region which we considered as an indication of their common origin. This proved to be true when our later analysis revealed strong evidence of phylogenetic interrelationship among these species.

The object of this study was to provide a cytological characterization of the members of this group and to determine their relation to one another and to those species with standard centromere region.

The controversy regarding the identity of *P. hirtipes* (Fries), and the taxonomic status of certain Southern and Central European *Prosimulium* species belonging to this group, added further incentive to a detailed chromosome analysis of each of these species.

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Material

Prosimulium is holarctic in distribution and consists of over 50 known species which are generally addicted to fast-running mountain streams. The first species of *Prosimulium* to be described was *Prosimulium hirtipes* (Fries) from Scandinavian Lapland (14). The same name was subsequently applied to forms from other parts of Europe (11, 22, 27) and the North American continent (28, 29, 31).

It became clear early in the cytological work (24) that the forms subsumed under *P. hirtipes* (Fries) included at least three common and several rare species in North America all of which were specifically distinct from the European *P. hirtipes*. Recently, the forms segregated as *P. hirtipes* 1, *P. hirtipes* 2, and *P. hirtipes* 3 in this laboratory have been erected as new species (*P. hirtipes* 1 = *P. fuscum* Syme and Davies, *P. hirtipes* 2 = *P. mixtum* Syme and Davies, and *P. hirtipes* 3 = *P. fontanum* Syme and Davies) on morphological grounds from cytologically pure material (30).

P. fontanum, the only North American form sharing the "transformed centromere" with the European *Prosimulium* species, is so far reported only from the granite portions of Ontario and Quebec (30). The larval samples for the present work were collected by Rothfels in 1957 from a rill going into the Ottawa River at the North Star Resort, on Highway 17 (4 miles west of Deep River, Ontario) and from two streams near Fox Point Post Office, Lake of Bays, Ontario). L. Davies sent a batch of larvae of this species in 1958 from a stream near Notch Road in the Gatineau about 8 miles north of Ottawa. Prepared slides of various populations of *P. fontanum* larvae, collected and processed by Rothfels and V. R. Basrur from Chalk River and Algonquin Park in 1955, also were included in the present cytological investigation.

TABLE I

Collection data of seven forms of *Prosimulium* showing date, location, sample size, and sex

Species	Collection date	Location	Total no.	Male	Female
Prosimulium fontanum	22.5.1955	Brewer Lake, Algonquin Park, Ont.	12	3	9
	25.5.1955	Brewer Lake, Algonquin Park, Ont.	6	2	4
	24.5.1955	Opeongo Lake, Algonquin Park, Ont.	16	3	13
	1.6.1957	Chalk River, Ont.	34	5	29
	2.6.1957	Fox Point Inn, Lake of Bays, Ont.	18	7	11
	19.6.1958	Notch Road, Ottawa, Ont.	16	3	13
P. hirtipes	24.4.1956	Swindale Beck, Westmorland, England	59	32	27
	30.4.1956	Swindale Beck, Westmorland, England	24	17	7
	18.4.1957	Swindale Beck, Westmorland, England	98	59	39
	1.5.1957	Inverness-shire, Scotland	26	9	17
P. inflatum	25.4.1957	Inverness-shire, Scotland	28	15	11
P. vigintiquaterni	3.5.1957	Jeseniky Mountain, Czechoslovakia	29	11	18
	28.5.1957	Jeseniky Mountain, Czechoslovakia	7	4	3
	30.5.1957	Jeseniky Mountain, Czechoslovakia	6	4	2
	27.6.1957	Jeseniky Mountain, Czechoslovakia	8	1	7
P. hirtipes arvernense	19.6.1958	Col de la Croix St. Robert, Central France	5		
P. inflatum aff. 1	8.6.1958	Lac d'Oredon, Haute (High) Pyrenees, France	24		
	8.7.1958	St. Moritz, Engadin Valley, Swiss Alps	20		
P. inflatum aff. 2	16.6.1958	Grenoble, French Alps	22		
	19.6.1958	Besse-en-Chandesse, Puy de Dôme region, Central France	18		
P. inflatum aff. 3	1953	Lunz, Austria	12		

On detailed re-examination of *P. hirtipes* from the European continent, Davies (7) found a common dark-legged North European form to be structurally identical with that described by Edwards (11) and Puri (22) in the name of *P. hirtipes* (Fries), while the South European forms differed and showed striking variation in larval and adult features; hence he proposed to restrict the name *P. hirtipes* (Fries) to the large, dark-legged North European species. Submitting to the strength of this argument, the larvae he sent from England and Scotland are referred to as *P. hirtipes* (Fries) in this report.

The distribution of this species has been confirmed in the Scottish Highlands, Northern England, North Wales, and the Scandinavian countries (7). For our cytological work, several batches of larvae were sent by L. Davies from Swindale Beck, Westmorland (England), and a batch of larvae from the River Fishie, Inverness-shire (Scotland).

Prosimulium vigintiquaterni Enderlein was erected as a species in 1929 (13), based upon the pupae Enderlein collected from Central Europe (Brandenburg, Günterberg, River Oder). Novak's observations (20) on larvae and pupae from Czechoslovakia supplement the information on the morphological aspects of this species. *P. vigintiquaterni* pupae possess 25 to 26 respiratory filaments as against 16 in *P. hirtipes*. Grenier (15, 16) described a rather similar form, also with 25 respiratory filaments, from Central France in the name of *P. hirtipes arvernense* Grenier. Our observations on *P. vigintiquaterni* and *P. hirtipes arvernense* substantiate Novak's conclusion (personal communication) that the two species are one and the same. However, in the text, the salivary gland chromosomes of the two "species" are treated separately. Larval samples of *P. vigintiquaterni* were sent by Novak from Furkota, Vysoke Tatry, and Jeseniky Mountain, in Czechoslovakia. A batch of immature larvae of *P. hirtipes arvernense* was sent by D. M. Davies from Col de la Croix St. Robert (16 miles from Besse en route to Mont Dore, near Banz), which provided a few "workable" slides.

Prosimulium inflatum Davies is a new species allied to *P. hirtipes* (7). The males of *P. inflatum* and *P. hirtipes* are virtually identical while the females are quite different in the structure of the genitalia and other morphological features (7). Specimens of all stages have been collected from various streams in England and Scotland (7). The material for cytological analysis comes from the stream on Carn Ban Mor Mountain, Inverness-shire (Scotland).

Several batches of *Prosimulium* larvae were sent to us by D. M. Davies from various streams in the Alps and the High Pyrenees (France). Cytologically these larvae could be orientated into two groups: the seven samples of larvae from the High Pyrenees form one group which we call *P. inflatum* aff. 1 and the samples from the French Alps and Central France (Table I) fall into another group which we propose to call *P. inflatum* aff. 2. E. Kunze-Mühl sent us prepared slides of certain species of black flies from the vicinity of Lunz, Austria, which she had assorted into four species on the basis of gross salivary gland chromosome features. Our observations revealed that 12 of these slides which according to Kunze-Mühl belong to species III (19), belong to a distinct form which we call *P. inflatum* aff. 3. There was a single slide in

the lot also marked species III which contained chromosomes unmistakably similar to those of *P. hirtipes arvernense.* We consider this slide to be of *P. hirtipes arvernense.*

The various forms here referred to as *P. inflatum* aff. 1, *P. inflatum* aff. 2, and *P. inflatum* aff. 3 have not yet received extensive morphological study. Their original definition is purely cytological. Although the larval morphology of *P. inflatum* aff. 1 and *P. inflatum* aff. 2 has since been studied (see page 567), any conclusion on the taxonomic status of these three forms is provisional and conditional.

Table I gives additional information pertaining to the collection of larvae from various countries.

Method

The larvae were dissected, fixed in 1:2 acetic alcohol, and air-mailed to us in small vials with sufficient amount of fixative, from various collection locales (Table I). When the larvae, delayed in transit, were transferred to water, their salivary glands formed a colorless jelly-like substance which was removed by employing the standard technique of this laboratory (3). The "clean" larvae were hydrolyzed in normal HCl and stained in leucobasic fuchsin according to the method of Rothfels and Dunbar (23).

The dry ice technique of Conger and Fairchild (6) was employed in making permanent slides. In order to avoid fading of the stain, the pH of the mounting medium ("euparal") was regulated with marble chips.

Camera lucida drawings of chromosomes were made in pencil (using a 20× eyepiece and 95× oil immersion objective) to illustrate the inversions, wherever necessary.

Microphotographs were taken on Kodak "Panatomic X" film with a Leica 35-mm camera fitted with an ipso adaptor, developed in "microdol", and printed on Kodabromide F4. Pencil drawings were photocopied on Kodak Micro-File film, using a Leica photocopying attachment.

Cytological Observations

General Features of the Polytene Chromosomes

Gonial metaphase plates in all seven forms consist of three pairs of metacentric chromosomes of which chromosome I is "V"-shaped and decidedly the longest. The remaining two chromosomes differ in their length and are "J"-shaped. The corresponding three polytene chromosomes in salivary gland nuclei give arm ratios which approximate those of metaphase chromosomes.

The gross features of the salivary gland chromosomes such as the number and comparative length of chromosome arms, morphological features of the expanded region, positions of nucleolus and Ring of Balbiani, and the degree of pairing of chromosome constituents are strikingly similar in all seven forms. A chromocenter which is not uncommon in black flies (18, 19, 24) is present in all except two species (*P. hirtipes* and *P. vigintiquaterni*) in this group.

Description of Standard and Transformed Centromere

Contrary to other black fly species studied in this laboratory, i.e. *Simulium vittatum* (23) and *Eusimulium aureum* (10) in which each chromosome has a prominent expanded region, most *Prosimulium* species have an expanded region only on chromosome I. Even this is poorly expressed in two species from Alaska (*P. onychodactylum* and *P. alpestre*). These two species excepted, all *Prosimulium* species we have examined have the long "cottony" type of expanded region as against the short "compact" type found in *Simulium*. The standard expanded region of chromosome I, as found in all but one North American species, is illustrated by Fig. 1. The characteristic length of the expanded region is about one-tenth of the total length of chromosome I. The centromere, the last band in section 19, is shoestring-like in appearance and is exactly in the middle of the expanded region. Section 19 of the short arm in the standard expanded region is characterized by the absence of bands, while section 20 on the nucleolar arm has four heavy heterochromatic bands near the centromere and a light gap followed by a group of heavy bands (Fig. 1). On the other hand, the most distinctive feature of the species which falls within the province of this paper is the much-exaggerated and "transformed" centromere region of chromosome I (Figs. 2, 3, 4). The expanded region in these forms extends a considerable length on either side of the centromere, rendering about one-fifth of the total length of chromosome I heterochromatic. Section 19′ of the short arm has three heavy heterochromatic bands which often form a 'blister" (Figs. 3, 4), and a group of light and dark bands which are close to the centromere (Figs. 2, 3, 4). Sections 20′ and 21′ have more bands than the corresponding sections in the standard centromere region. The difference between the two kinds of centromere regions is so striking that all slides could be categorized unequivocally into the respective groups taking the centromere features alone into consideration. Furthermore, within the group with elaborated centromere region, in all except one species the characteristic heavy bands and their respective positions are unmistakably similar. The exception concerns a minor change in *P. fontanum* which is readily interpreted as an inversion (*IL-1*) compared with the other species in this group. Abortive attempts to compare the bands in the expanded region of these forms with those of standard centromere region leave us in doubt about their homology. For this reason, we speak of the change as "centromere transformation". And, since this change involves significant morphological specificity all species carrying "transformed" centromere region on chromosome I are considered to form a natural group.

Distinguishing Features and Landmarks

Apart from the relative lengths of chromosome arms and the "transformed centromere" region described above, a few other specific features and landmarks enable easy recognition of individual chromosomes and chromosome arms.

The short arm of chromosome I has the "3 heavy groups" which in the standard sequence is at the base of the short arm (Fig. 7), close to the expanded

region (24). The long arm of chromosome I has a nucleolus which in Feulgen-stained nuclei appears as a non-stained gap (Fig. 26).

Unlike some of their relatives in North America, *Prosimulium* species from Europe show an expanded region even on chromosome II. The short arm of chromosome II has a lightly stained and "puffed" region which maintains a definite and constant position near the centromere (Fig. 35). This "puffed" region in black flies is referred to as "Ring of Balbiani" (23) because of its similarity with those structures in chironomids (4). The prominent landmark on the long arm of chromosome II, the "group of 5" (24), is at the base of IIL (Fig. 47). The "para Balbiani", and the five distinct bands (26), form another landmark on IIL (Fig. 52).

There is no distinct expanded region in chromosome III. There is, however, a heterochromatic band (or group of bands) which, because it displays "ectopic pairing" with the centromeres of other chromosomes, is considered to be the centromere (Fig. 64). The short arm of chromosome III begins with a "frazzle" (24), where the bands are frayed out and disorganized (Fig. 59). The subterminal "blister" and the "2 heavy bands" associated with it form convenient landmarks on IIIS as does the "shield and triad" (24) on IIIL (Fig. 68).

Chromosome Maps and Plate Nomenclature

For the cytological analysis, the standard sequence of *P. mixtum* is chosen as universal standard for *Prosimulium*, except for IIIL for which the sequence

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Plate I

Figs. 1–6. Expanded region and chromocenter.

Fig. 1. Chromosome I, expanded region (*P. mixtum*).*
Fig. 2. Chromosome I, expanded region (*P. fontanum*).†
Fig. 3. Chromosome I, expanded region (*P. hirtipes*).†
Fig. 4. Chromosome I, expanded region (*P. inflatum*).†
Fig. 5. Chromosome I, expanded region (*P. fontanum*).‡
Fig. 6. Chromocenter (*P. fontanum*).‡

Abbreviations: C, centromere; CH, chromocenter.

*×2500.
†×1400.
‡×1050.

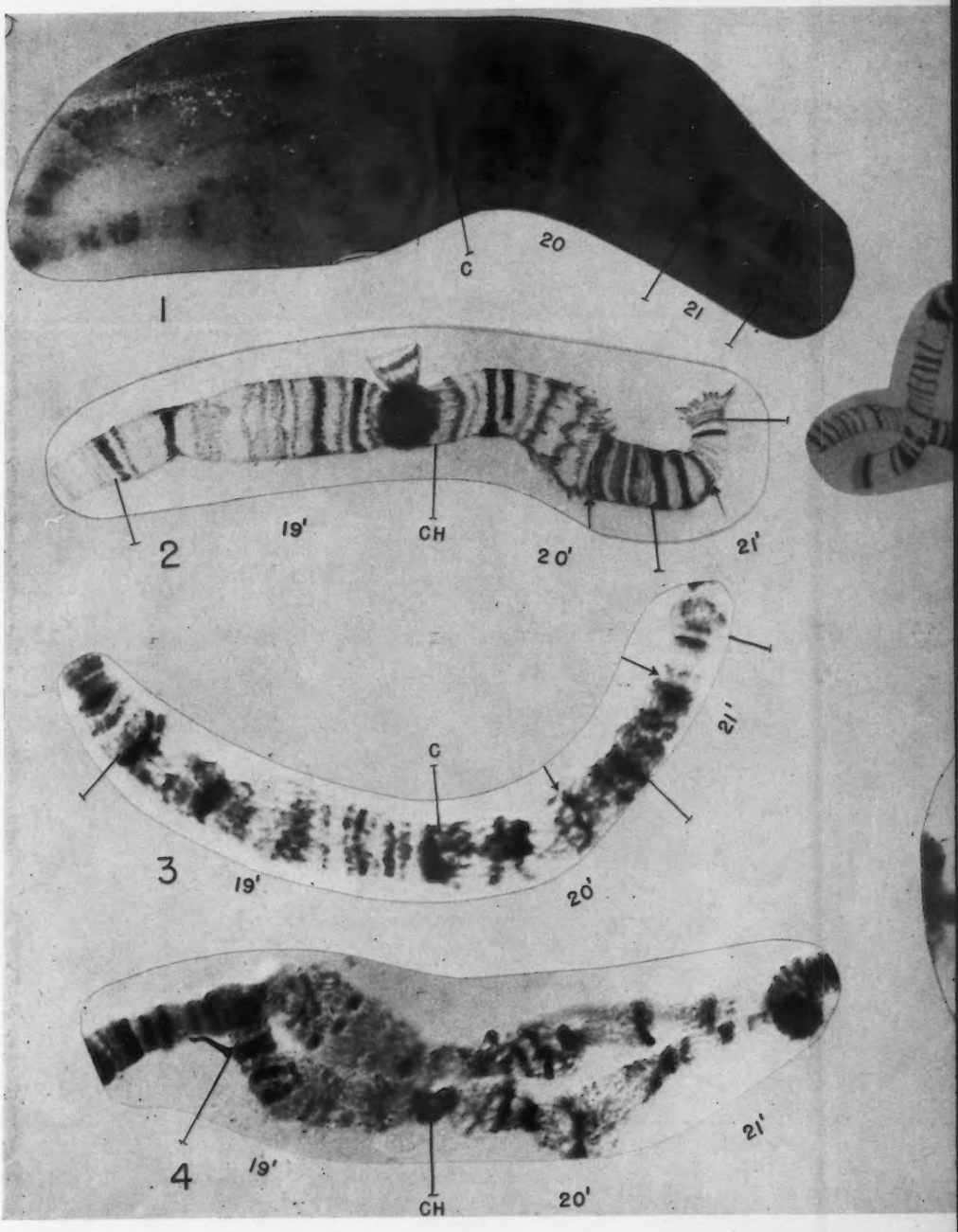
Plate II

Figs. 7–24. Chromosome I short arm.

Fig. 7. Sections 17–19 (*P. mixtum*).*
Fig. 8. IS-10/IS-10 (*P. hirtipes*).‡
Fig. 9. IS/IS-10 (*P. hirtipes*).†
Fig. 10. Sections 14–17 (*P. mixtum*).*
Fig. 11. IS/IS-19 (*P. inflatum* aff. 3).†
Fig. 12. IS-14,17 (*P. vigintiquaterni*).†
Fig. 13. IS/IS-14.15.16 (*P. vigintiquaterni*).†
Fig. 14. Sections 9–14 (*P. mixtum*).*
Fig. 15. Sections 1–12 (*P. mixtum*).*
Fig. 16. IS/IS-18 (*P. inflatum* aff. 1).†
Fig. 17. *IS-18* (*P. inflatum* aff. 2).†
Fig. 18. The "transformed centromere" region (*P. hirtipes*).†
Fig. 19. Sex-determining inversions (*P. fontanum*).‡
Fig. 20. *IS-8.9* (*P. hirtipes*).‡
Fig. 21. IS/IS-13 (*P. hirtipes*).†
Fig. 22. IS/IS-12 (*P. hirtipes*).†
Fig. 23. IS/IS-11 (*P. hirtipes*).†
Fig. 24. *IS-1* (*P. fontanum*).†

Abbreviations: "3", the "3 heavy groups".

*×2900.
†×1600.
‡×1200.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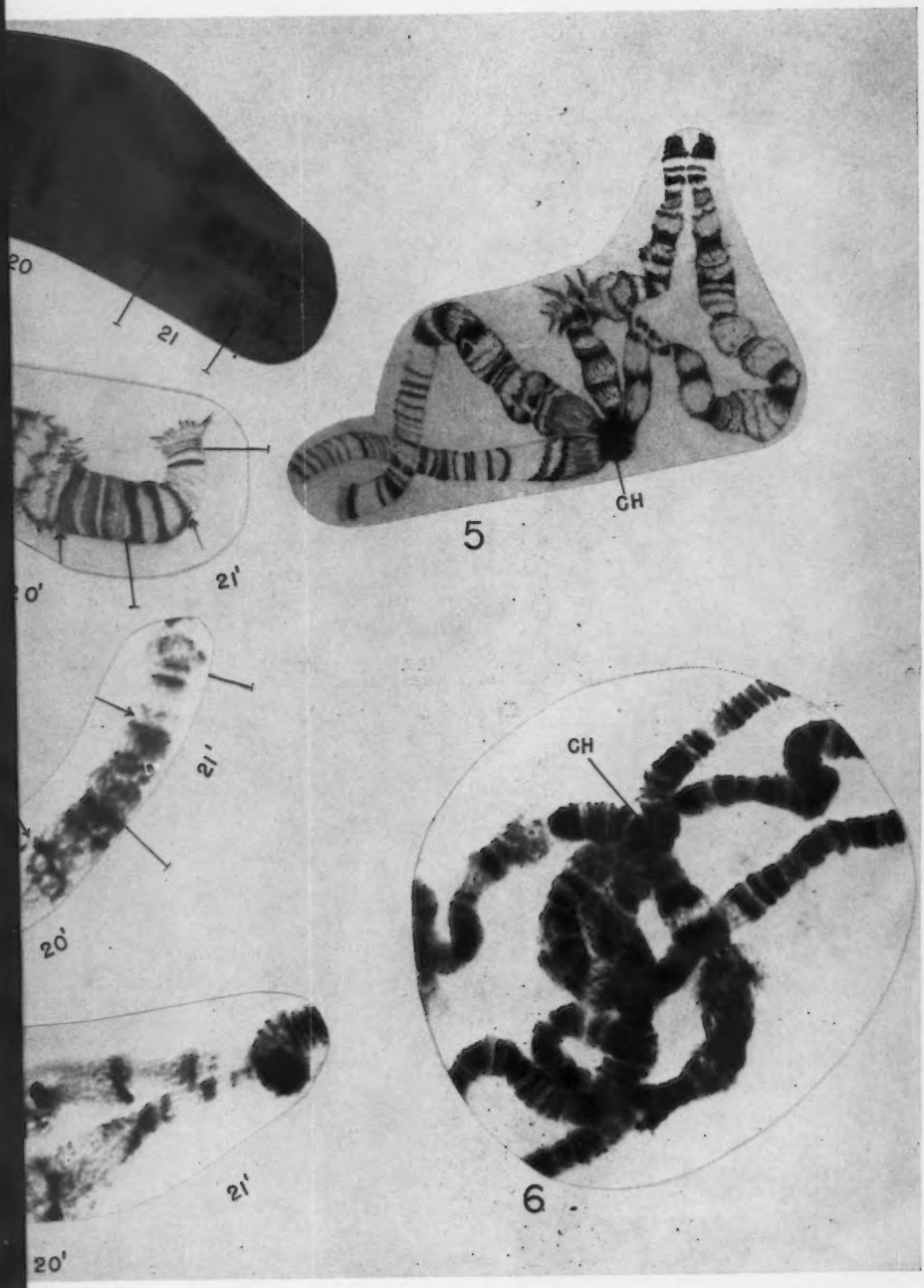
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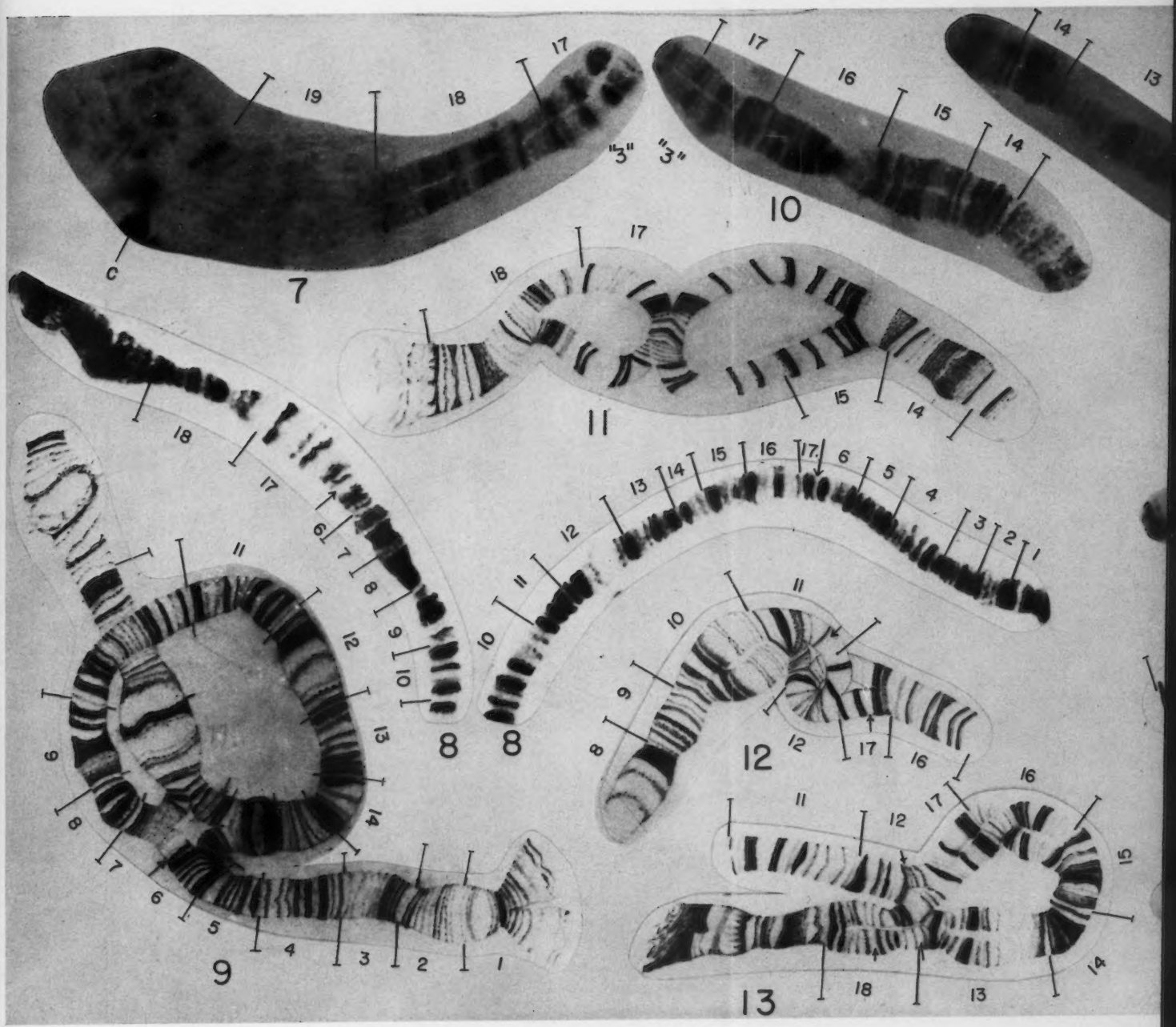
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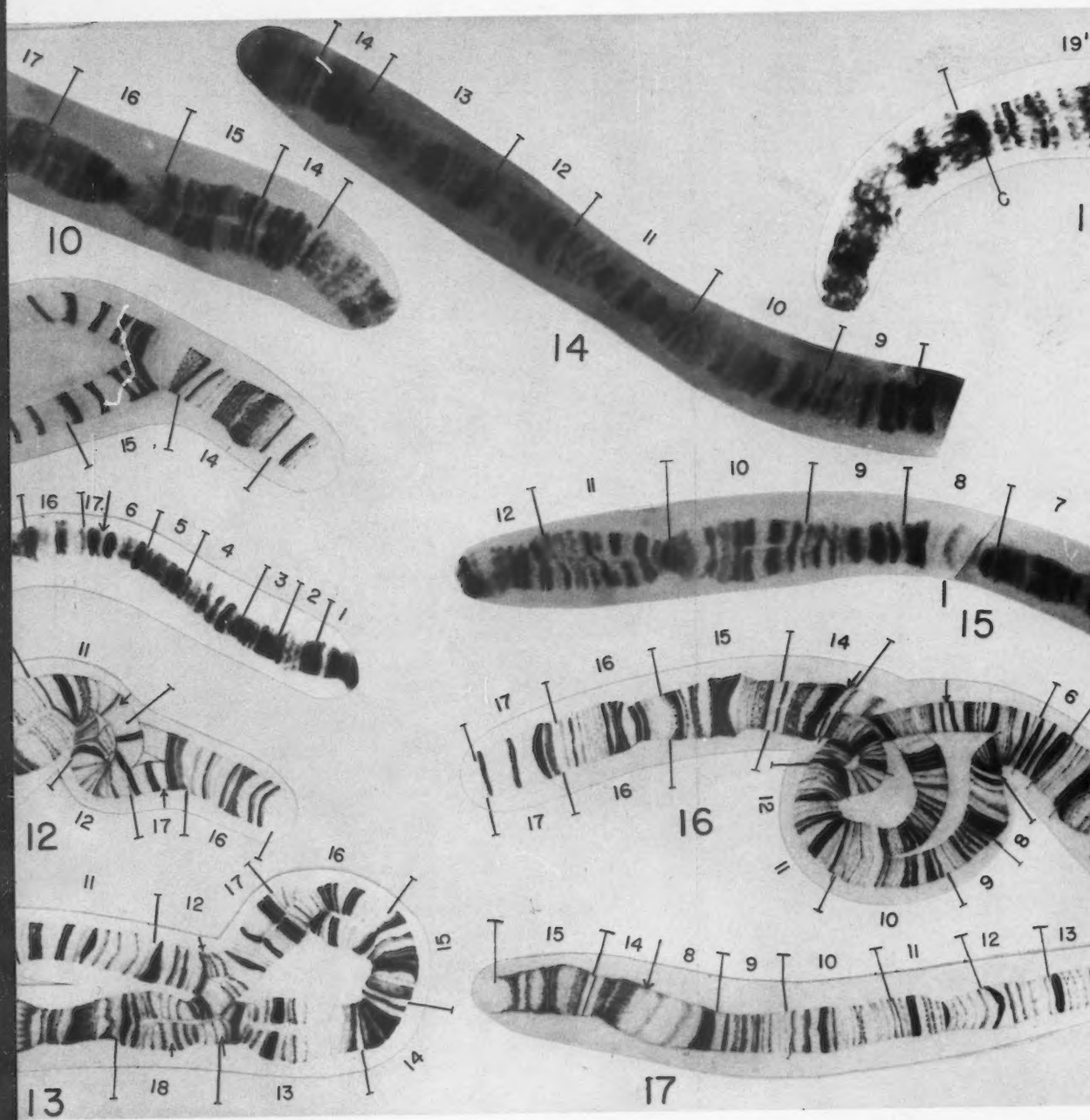
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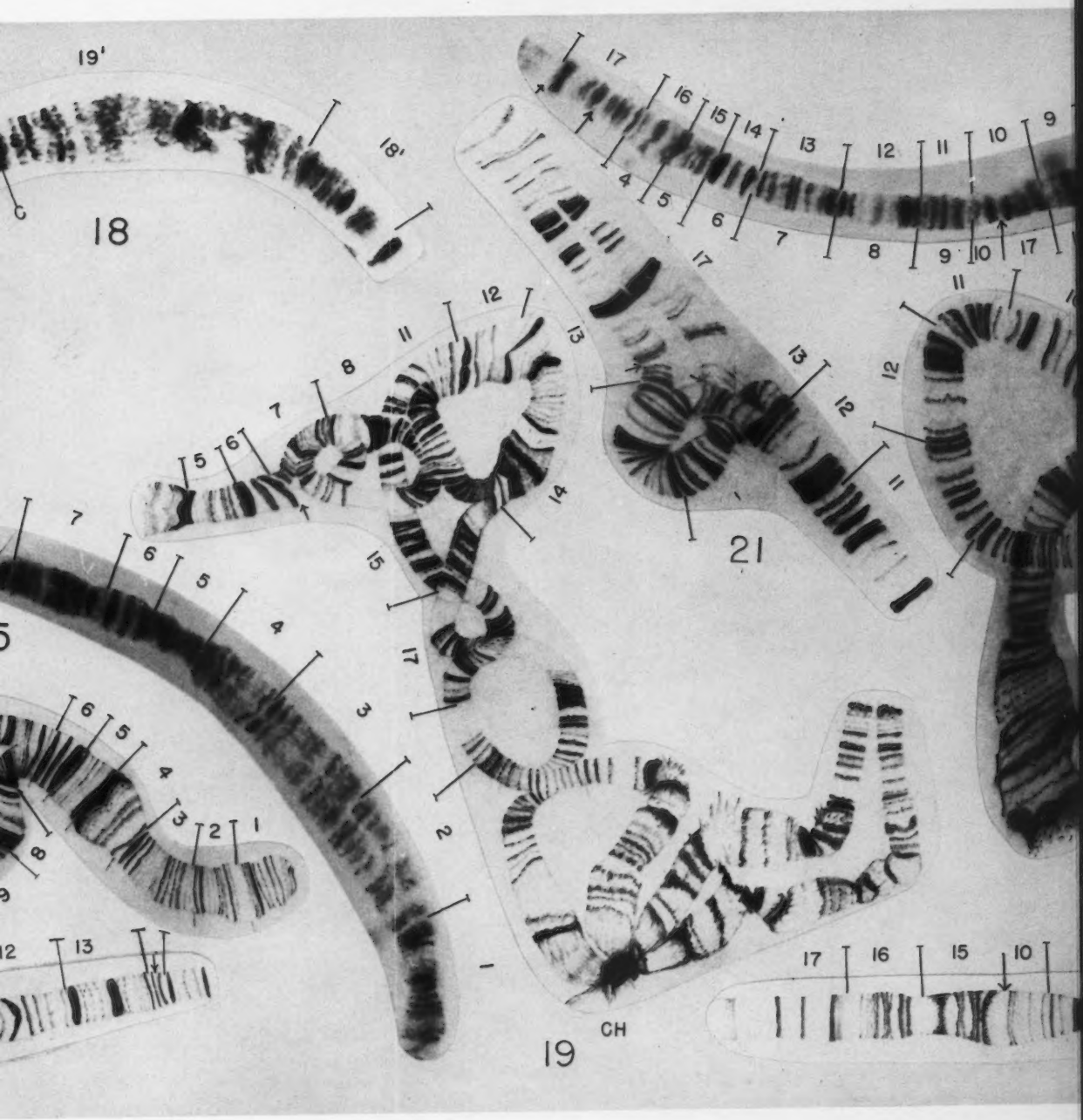
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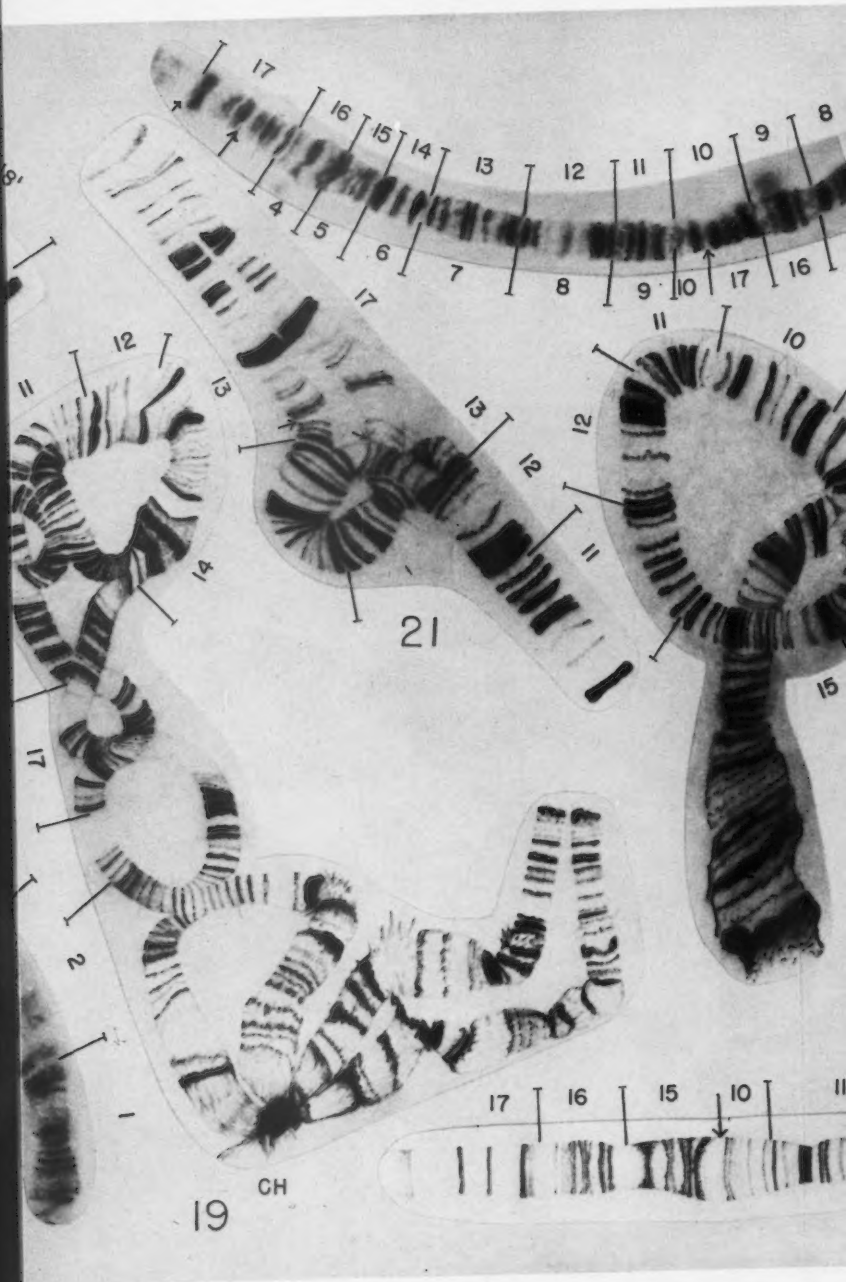
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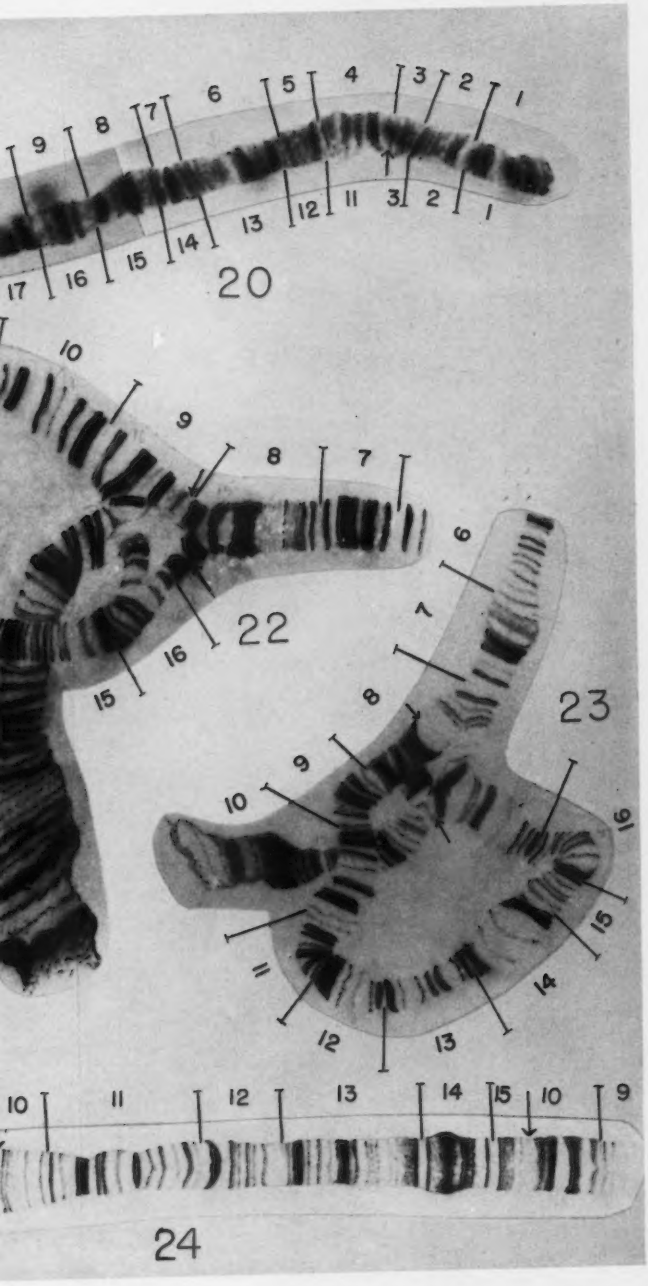
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22
23
24

of *P. fuscum* is considered to be more central. In the preparation of the maps the entire complement is divided into 100 sections of approximately equal length. The sections are numbered commencing from the end of the short arm of chromosome I, ascending to 44 in the last segment of the long arm. Similarly, the sections of the second and third chromosomes are numbered from 45 to 74 and 75 to 100, respectively. The section limits and section numbers of standard maps are retained in all forms in which the standard sequence is observed. Where a chromosome arm differs from standard by one or more steps, the sections are numbered on one side (generally below) in terms of the standard sequence in order to indicate the rearrangement of segments. In addition, the sections are given consecutive numbers (generally above) in order to facilitate the description of floating inversions superimposed on interspecific changes. The species which differ considerably from the universal standard are thus described by a "double standard": the genus standard (universal standard) below, and the species standard above, in the map. For convenience of comparison, in the tables the floating inversions which are many steps removed from standard are represented with respect to the species standard (in bracket) as well as to the universal standard. The gene sequence of chromosome arms presented in the text are always in relation to the standard (universal) arrangements of the respective arm.

The transformed segments in the centromere region of chromosome I are numbered 18′–21′ as against 18–21, in the standard. Maps of entire arms or of segments of chromosome arms of altered orientation only are presented in the plates. Uniformly conservative chromosome arms like IIIS and IIIL are represented only by standard maps, accompanied by drawings of floating inversions in each species. While referring to the break points, the letters "p", "c", and "d" are used to indicate the "proximal", "central", and "distal" one-thirds of sections. These letters only indicate the approximate position of break points in a segment while the actual break points are indicated by arrows on chromosome maps and drawings. The order of these letters follows the direction in which the section numbers proceed. The break points which fall between two sections are symbolized by the numbers of the two sections separated by a stroke, as in 49/50. For example: 30–37c. 50/49–37c.49/50–56 indicates a simple inversion with one break in the center of segment 37 and the other at the boundary between segments 49 and 50.

The inversions on a given chromosome arm, interspecific (in italics) and floating, are numbered consecutively from one in the order in which they are first mentioned in the text.

A cytological summary of each species is given in the form of an idiogram along with the detailed chromosome description. Following the conventions of this laboratory, idiograms are based on the data (Table II) obtained by measuring camera lucida linear drawings of five well-stretched nuclei of each species and converting the measurements into per cent total complement lengths (2, 3, 9, 10, 24, 33). Text-fig. 1 explains the conventions used in

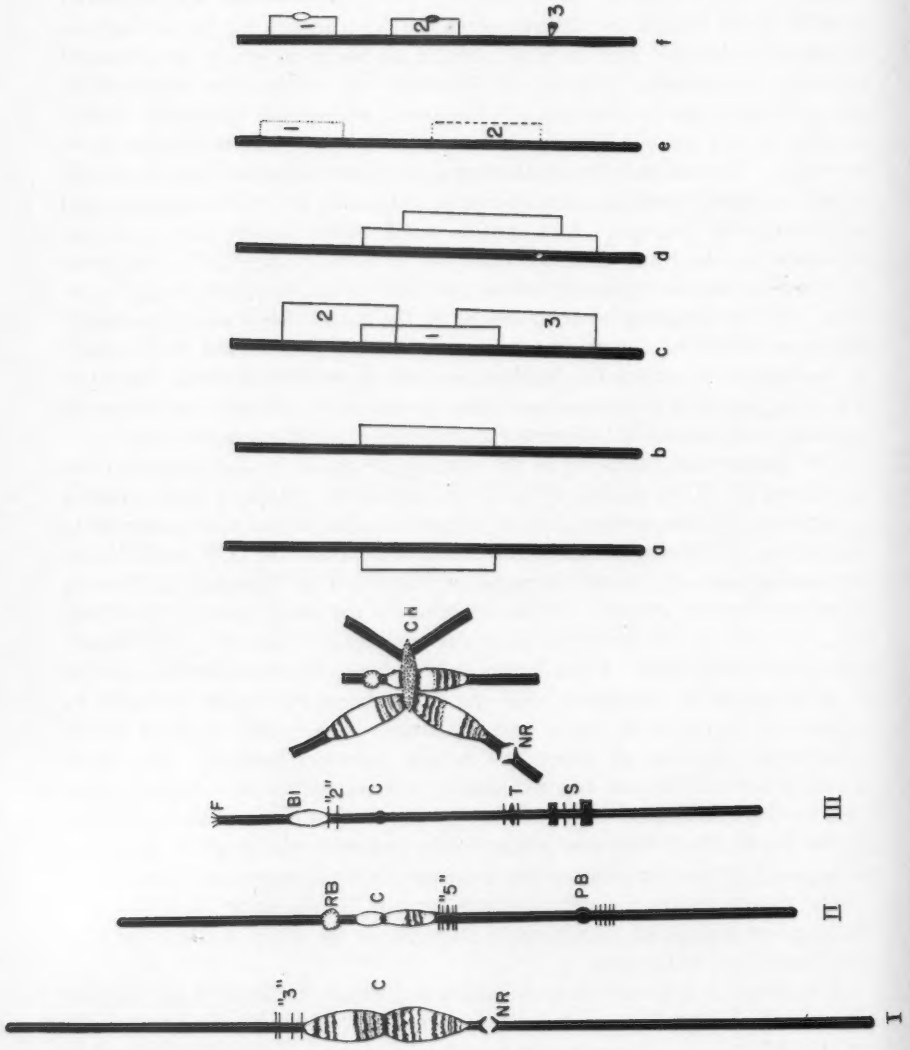
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TABLE II

Per cent total complement length (% TCL) of seven forms of *Prosimulium*
(Mean of five nuclei taken in each case)

	Chromosome I			Chromosome II			Chromosome III	
	IS	IL	IL	IIS	IIS	IIL	IIIS	IIIL
Species	E—C	C—N	N—E	E—RB	RB—C	C—E	E—C	C—E
Prosimulium fontanum	21.08	5.27	19.38	9.46	2.44	16.88	7.19	17.83
P. hirtipes	19.38	5.62	20.28	10.34	1.53	17.92	8.36	16.61
P. inflatum	21.25	5.54	18.49	9.20	2.50	18.58	7.87	16.58
P. vigintiquaterni	18.70	5.81	17.06	9.94	2.94	19.99	7.93	18.01
P. inflatum aff. 2	20.95	5.67	18.71	9.49	2.60	18.42	7.69	16.97
P. inflatum aff. 3	21.28	5.29	18.99	9.18	2.62	19.19	7.66	17.49

NOTE—E: end; C: centromere; N: nucleolus; S: short arm; L: long arm; RB: Ring of Balbiani.

constructing the idiograms (Text-figs. 2–7). The chromosomes are represented according to their length from left to right, their centromeres orientated in the equatorial plane, and the short arm facing upward while the long arm points down. The prominent landmarks are emphasized in their respective places on each chromosome arm. The approximate positions and dimensions of inversions are shown by brackets: the interspecific inversions on the left and the intraspecific inversions on the right side of the individual arms. Repeats and extra heterochromatic pieces are shown as bulges (plain in the case of repeats and solid when heterochromatic) on the brackets. Inversions on chromosomes which function as genetic *X* and *Y* chromosomes are indicated by dotted and broken lines respectively. "Simple" inversions are represented by brackets with their extremities touching the chromosome arm and the "overlapping" inversions, with one of their extremities resting on the bracket representing the first inversion step. In the case of "included" inversions, both the limbs of a bracket, representing the shorter inversion, rest on the bracket which represents the longer inversion.

The break points that fall on segments which are already displaced by an inversion step are indicated by broken lines in Text-figs. 8–13.

Text-fig. 1. Model idiogram explaining the conventions used for landmarks and rearrangements. I, chromosome I; II, chromosome II; III, chromosome III; C, centromere; "3", the "3 heavy groups"; NR, nucleolar region; RB, Ring of Balbiani, "5", the "group of 5"; PB, para Balbiani; F, frazzle; Bl, blister; "2", the "two heavy bands"; T, triad; S, shield; Ch, chromocenter.

(*a*) interspecific inversion (simple).
(*b*) intraspecific inversion (simple).
(*c*) overlapping inversions: 2 overlaps 1 on the map,
3 overlaps 1 on one constituent.
(*d*) included inversion.
(*e*) inversions on sex chromosomes: 1, inversion on *X*;
2, inversion on *Y*.
(*f*) rearrangements involving repeats and extra heterochromatin:
1, repeat in an inversion;
2, extra heterochromatin in an inversion;
3, extra heterochromatin.

Analysis of the Banding Pattern

Results of the detailed analysis of chromosome complements, given under sections devoted to each species, cover (1) interspecific or "fixed" inversion differences from standard and (2) intraspecific or "floating" inversion differences. The cytological sex-determining mechanisms are discussed along with the floating inversions.

Prosimulium fontanum Syme and Davies

The cytological description of *P. fontanum* presented here is based on the detailed analysis of larval populations from several locations in Ontario (Table I). Feulgen-stained salivary gland chromosomes of this species appeared to be generally fainter and thinner than those of *P. mixtum*. The massive chromocenter, holding the six chromosome arms together, is seldom broken in slide preparation (Fig. 6). The expanded region of chromosome I is enormous, in keeping with the group characteristic of the forms discussed in this paper.

Standard IS, shown by Figs. 7, 10, 14, 15, consists of 19 sections, sections 18 and 19, which we call 18′ and 19′ in this group, being in the expanded region. In *P. fontanum* the last band of section 19, representing the centromere, is eclipsed by the heterochromatic chromocenter. The striking landmark, the "3 heavy groups", is in section 17, as in standard (Fig. 7) but the "cup and saucer" (26) which in standard is in section 14 (Fig. 10) has changed its position in all females through a simple inversion, *IS-1*. The gene sequence of IS in *P. fontanum* with respect to the standard can be represented as follows:

1–10*c*. 15*c*–10*c*.15*c*–19.

One break point of inversion *IS-1* falls within the light central region of section 15 (15*c*) and the other falls close to the finer bands in the central one-third of section 10, as shown by arrows in Fig. 24. In males the same inversion is found, but the situation is complicated by the fact that in *P. fontanum*, IS is found as the sex-determining arm, and in males additional rearrangements have occurred in one constituent which evidently is the genetic *Y* chromosome (see above).

Standard IL is divided into 25 sections, commencing with 20 and ending in 44 (Figs. 26, 30, 31, 32). The first two sections, 20 and 21, of standard IL (Fig. 26) constitute the remainder of the expanded region and are numbered 20′ and 21′ in *P. fontanum* (Fig. 2). Bands in this region are often deeply stained and highly disorganized. The nucleolar region, represented by fine broken bands in Feulgen-stained nuclei, is situated in section 22, as in standard (Fig. 26); but a rearrangement, *IL-1*, places the heterochromatic bands in segment 20′*c*–21′*c* in reverse orientation (Fig. 3) as compared with this region of the European forms in this group.

Standard IIS has 13 sections (45–57), the last band of section 57 representing the centromere (Figs. 35, 40, 41). In *P. fontanum* the gene sequence of IIS is altered by an inversion *IIS-1*, which could be represented as:

45–49/50. 55*c*–50/49. 55*c*–57.

Thus, *IIS-1* extends from the section limit of 49 to the central one-third of section 55, and inverts the orientation of the "double bulge" (26) which in standard is in section 53 (Figs. 35, 38, 40). The "glassy band", 48*c* (Figs. 38, 41), is in its standard position (26) in the predominant gene sequence of *P. fontanum* IIS.

Standard IIL starts from section 58 and ends in 74 (Figs. 47, 52, 53, 54). A slightly expanded region extending from the centromere to the middle of section 58 is present on IIL (Fig. 47). The "group of 5", identified by the central heavy band with two fine bands on either side of it, is at the distal one-third of section 59 (Fig. 47), while the "capsule" (26), a compound of two groups of heavy bands, occupies the proximal one-third of section 64 (Figs. 47, 52). The "para Balbiani" and associated bands (26) cover the whole of section 66 (Fig. 52). IIL of *P. fontanum* is band for band identical with standard.

IIIS is the shortest of chromosome arms, with only 8 sections (75 to 82). The extreme end of section 75 is frayed out to give a "frazzle" appearance (Fig 59). Section 78 accommodates the "blister" which is followed by the "2 heavy bands". The heterochromatic band "C", representing the centromere (Fig. 64), terminates section 8. There is no interspecific inversion on IIIS of *P. fontanum.*

Standard IIIL runs into 17 segments, sections 83–100 (Figs. 61, 62, 63). An interspecific rearrangement *IIIL-1*, identical with that in *P. mixtum* (24), changes the orientation of the "shield and triad" in *P. fontanum*, from standard. One of the break points of this inversion falls at the section limit 87/88 (Figs. 64, 68, 69) while the other separates the first of the closely packed bands at 94*p* (Fig. 72). This inversion brings the IIIL band sequence of *P. fontanum* to:

83–87/88.94*p*–88/87.94*p*–100.

In addition, IIIL of *P. fontanum* shows another small deviation from standard: one of the two fine bands, 92*d*, associated with the "shield", is consistently much denser in *P. fontanum* (Fig. 69). Although this arm was critically examined in nuclei from many slides, it was not possible to attribute this change to a rearrangement. Since we can account for every band on IIIL, the dense band in 92*d* of *P. fontanum* must be the result of a modification, possibly a doubling, of a single band.

IS of *P. fontanum* exhibits a complex configuration in males. Out of 102 larvae observed, 23 were males all of which showed a chain of loops extending from section 6/7 to section 20′ of the nucleolar arm (Fig. 19). Analysis of these loops showed that one constituent has the species (*P. fontanum*) standard sequence. The gene sequence of the other constituent is as shown below:

1–6/7.10/11–11*c*.8*d*–6/7.11/10–8*d*.11*c*–15*p*.15*p*–16*d*.
18′/17–16*d*.18′/19′–17/18′.

In addition, in this constituent, section 19′ of IS and 20′ of IL are also rearranged, although the nature of this rearrangement is difficult to under-

TABLE III

Test for conformity with the Hardy-Weinberg distribution of IIS-2, IIL-3, IS-10, IS-11, and IL-2

Combination	IIS-2			IIL-3			IS-10			IS-11			IL-2		
	S/S	S/2	2/2	L/L	L/3	3/3	S/S	S/10	10/10	S/S	S/11	11/11	L/L	L/2	2/2
Observed	26	23	4	25	20	5	64	73	15	12	9	2	12	8	2
Expected	26.4	23.1	4.5	24.5	21	4.5	69.1	68.1	19.7	11.6	9.3	1.8	11.6	8.7	1.6
χ^2		.0005			.05			.38			.09			.56	
P		$>.99$			.85			.55			.75			.45	

stand because of excessive heterochromatization in this region. The three heterochromatic bands, which in the standard constituent of *P. fontanum* (and also in all other species in this group) are in 19*p*, seem to be shifted to the vicinity of section 21′ on the nucleolar arm (Fig. 5). Apart from these bands, no other band in the rearranged segment (IS–2) could be homologized. The inverted segments 18′/17–16*d* and 18′/19′–17/18′ seem to be the results of two independent inversions IS-3 and IS-4 respectively, sharing one break point, 17/18′. An unchanged segment 15*p*–16*d* separates the inverted segments (IS-3 and IS-4) from the complex loop which precedes section 15. The rearrangement involving 6/7–15*p* could be derived in three steps from the standard sequence of this species (*IS-1*). According to the scheme which is most economical of inversion steps, the first step will be a rearrangement involving the segment 10/11–11*c* (IS-5). The next step will invert the segment 8*d*–11*c* (IS-6). The third step, involving the segment 6/7–10/11 (IS-6) will bring the gene sequence to that observed in the inverted constituent.

These inversions were seen exclusively in males. The absence of females with, and of males without, this complex inversion indicated to us that inversions IS-2.3.4.5.6.7 are associated with the cytological sex-determining mechanism. The absence of homozygotes for this inversion in either sex and the occurrence of similar cytological sex-determining mechanism in other black flies (24) lent support to this interpretation. These observations indicate that in *P. fontanum* both *X* and *Y* differ from standard IS in a simple inversion, *IS-1*, and that *Y* has further deviated from standard in six rearrangements IS-2.3.4.5.6.7, the nature of IS-2 remaining not clearly understood.

A floating inversion IIS-2 was seen in *P. fontanum* in great frequency (Table III). The break points of this inversion, shown by arrows in Figs. 38, 39, fall in 48*d* and 51*p* (Table VII). This inversion overlaps the interspecific inversion *IIS-1*.

No rare floating inversion was detected in any of the six populations studied.

The foregoing cytological details of *P. fontanum* are graphically shown in the idiogram (Text-fig. 2).

Since the total number of sections and the positions of the prominent landmarks (i.e. nucleolar organizer, Ring of Balbiani, etc.) on respective chromosome arms are the same as in *P. fontanum*, the cytological descriptions of the other species (*P. hirtipes*, *P. vigintiquaterni*, *P. inflatum*, and related species) are restricted to their differences, interspecific and intraspecific, from standard.

Prosimulium hirtipes (Fries)

Sample populations of this species from England and Scotland generally provided us with good cytological preparations. The bands of *P. hirtipes* are highly telescoped and the arms usually appear to be shorter than those of *P. mixtum*. Unlike *P. fontanum*, *P. hirtipes* has no chromocenter. The centromere region of chromosome I, as in *P. fontanum*, is highly heterochromatized (Fig. 18).

Text-fig. 2. *Prosimulium fontanum.*

Compared to standard, IS of *P. hirtipes* has a rearranged gene sequence which could be represented as follows:

1–3*c*.10/11–17/18′.10*p*–3*c*.11/10–10*p*.18′–19′.

This gene arrangement could be derived from standard in two inversion steps. The first step, *IS-8*, with breaks at 10*p* and 17/18′ and the second step, *IS-9*, inverting the segment 10/11–3*c* will bring the gene sequence to that of *P. hirtipes* IS.

The IS map of *P. hirtipes* is numbered from 1 to 19′ above in Fig. 20. The numbers below show the rearrangement of segments in terms of the universal standard.

The expanded region extending to IL differs from that of *P. fontanum* in not having *IL-1* (Figs. 2 and 3). The segment 20′*c*–21′*c* is in the same orientation in all European forms as in *P. hirtipes* (Fig. 3).

The band sequence of IIS in *P. hirtipes* (Figs. 34, 36, 46) is as follows:

45–48*c*.55*c*–48*c*.56*p*–55*c*.56*p*–57.

This sequence could be derived from standard in two independent inversion steps. One of these, *IIS-3*, involves three-fourths of the length of the arm extending from 48*c*–55*p*. The other inversion, *IIS-4*, involves a small segment, 55*c*–56*p* (Fig. 34). The break points in 55*c* seem to be coincident or near-coincident. The exact break points are hard to locate as the bands in 55*c* are faint and without distinctive individuality. The arrows in Figs. 34, 36, and 46 show the break points of the two inversions.

The band sequence of IIL in *P. hirtipes* could be represented as shown below:

58–64*p*.71/70–64*p*.70/71–74.

This arrangement could be achieved through a simple inversion, *IIL-1* (Table VIII). *IIL-1* changes the orientation of the "para Balbiana" and associated bands (Fig. 55).

IIIL of *P. hirtipes* is standard with the "shield and triad" orientated as in *P. fuscum*. The presence of this grouping in section 93 (Fig. 71) close to the heavy band, 94*p*, is characteristic of all European species dealt with in this paper.

A common floating inversion (IS-10) involving segment 3*c*–13*c* occurs in IS of *P. hirtipes* from England. The break points of this inversion are shown in Fig. 9. Table IV shows the gene sequence of IS-10, with respect to

TABLE IV

Description of inversions on the short arm of chromosome I

P. fontanum	
IS-1	1–10*c*. 15*c*–10*c*. 15*c*–19′
IS-2.3.4.5.6.7 (*Y*)	1–6/7.10/11–11*c*.8*d*–6/7.11/10–8*d*.11*c*–15*p*.15*p*–16*d*.18′/17′–16*d*.18′/19′–17/18′.19′
P. hirtipes	
IS-8.9	1–3*d*.10/11–17/18′.10*p*–3*d*.11/10–10*p*.18′–19′
IS-10	1–3*c*.10/11–13*c*.3*d*–10*p*.18′/17–13*c*.3*d*–3*c*.11/10–10*p*.17/18′–19′ (1–6/7.16*c*–7/6.16*c*–19′)*
IS-11	1–3*c*.10/11–15*c*.11/10–10*p*.3*c*–10*p*.18′/17–15*c*.17/18′–19′ (1–8*c*.18′/17–8*c*.17/18′–19′)
IS-12	1–3*c*.10/11–15/16.3*c*–10*p*.18′/17–16/15.11/10–10*p*.17/18′–19′ (1–8/9.16*p*-9/8.16*c*–19′)
IS-13	1–3*c*.10/11–17/18′.10*p*–7*p*.3*c*–7*p*.11/10–10*p*.18′/17–19′ (1–13*d*.16*c*–13*d*.16*c*–19′)
P. vigintiquaterni	
IS-14; IS-15 (*X*)	1–12*d*.18′*p*–12*d*.18′*c*–18′*p*.18′*c*–19′
IS-16 (*Y*)	1–16/17.17*p*–17/16.17*p*–19′
IS-14.17	1–11*d*.17*p*–11*d*.12*d*–17*d*.17*p*–12*d*.17*d*–19′
P. inflatum group	
IS-18	1–8*p*.14*p*–8*p*.14*p*–19′
P. inflatum aff. 3	
IS-19	1–16*p*.18/17–16*p*.17/18′–19′

*Description in parentheses is with respect to the species standard (see page 529).

TABLE V

The chromosome constitution of males and females of *Prosimulium hirtipes* with respect to inversion IS-10

Sex	S/S	S/10	10/10
Male	31	78	0
Female	26	20	15

NOTE: Eleven larvae were too immature to be sexed.

the universal and to the species standard. IS-10 in homozygous condition (Fig. 8) was seen only in females (Table V). Its relationship with the cytological sex-determining mechanism is discussed later.

The long arm of chromosome III has two floating inversions: IIIL-2 and IIIL-3. IIIL-2 extends from 87*d*–88*d* (Fig. 60) and appeared twice in 181 forms. IIIL-3 extends from 93/92 to 92/91 and carries an extra heterochromatic block. Since all standard bands are represented in the reversed constituent (Fig. 75), the heterochromatic piece appears to be an extra segment, probably formed by the duplication of a small segment. The individuals bearing this discrepancy on IIIL were re-examined to see if IIIL-3 were limited to either sex. Two males and three females showed this inversion, which proved that it plays no significant role in sex-determination.

Another heterochromatin-associated non-conformity (IIIL-4) was seen at the extreme end of IIIL in 14 larvae where, in the heterozygous condition, the two constituents flare apart into an inverted "Y", with unequal ends (Fig. 76).

PLATE III

FIGS. 25–33. Chromosome I, long arm.

FIG. 25. Centromere non-conformity and IL/IL-6 (*P. inflatum*).†
FIG. 26. Standard IL (*P. mixtum*).†
FIG. 27. IL/IL-5 (*P. hirtipes*).*
FIG. 28. IL/IL-2 (*P. hirtipes*).†
FIG. 29. IL/IL-3.4 (*P. hirtipes*).†
FIG. 30. Sections 22–36 (*P. mixtum*).†
FIG. 31. Sections 34–44 (The bracket shows the segment involved in inversion IL-7 of *P. inflatum* aff. 1) (*P. mixtum*).†
FIG. 32. Sections 23–34 (*P. mixtum*).†
FIG. 33. IL-8 (*P. inflatum* aff. 2).†

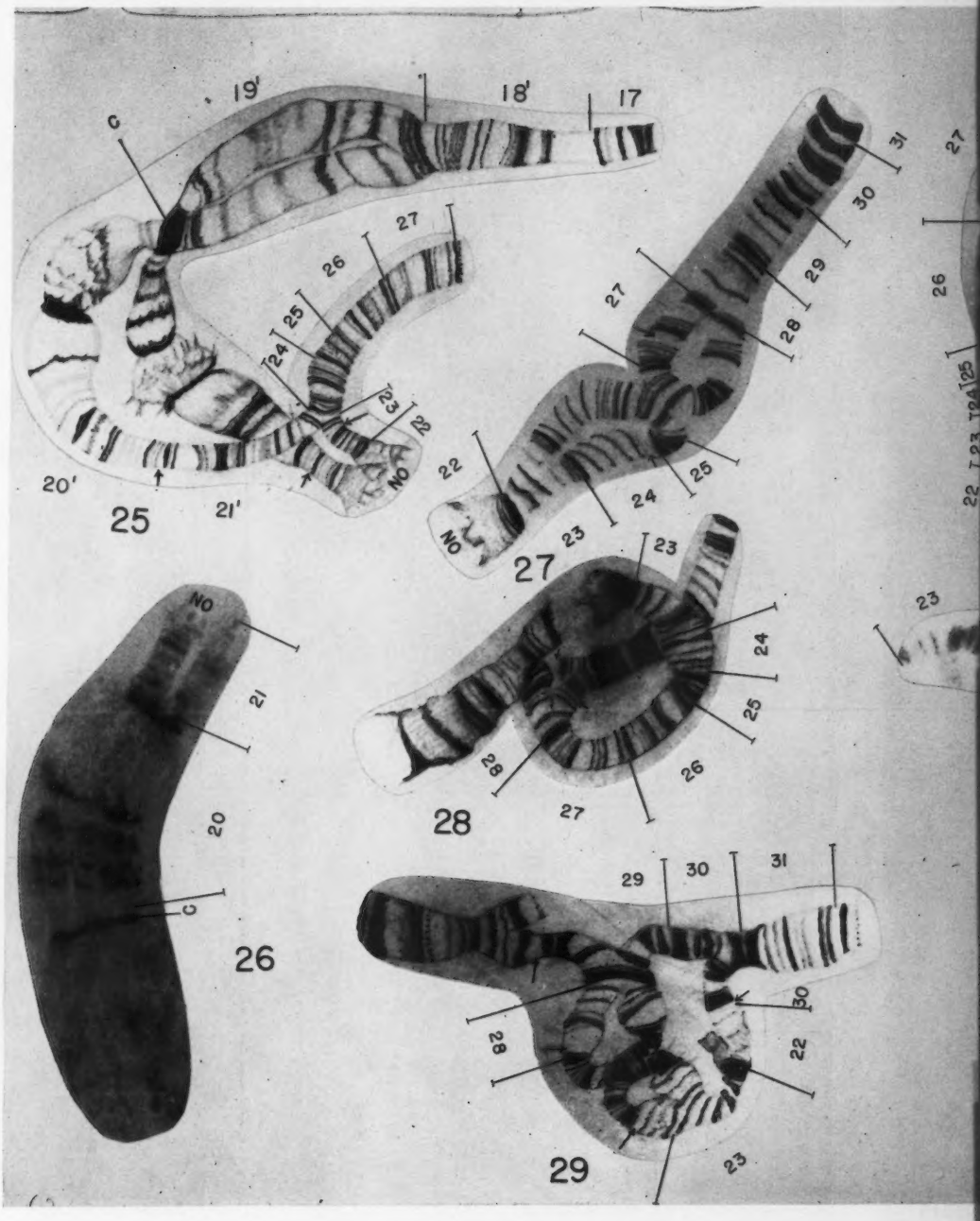
ABBREVIATIONS: NR, "nucleolar region".
*×2300.
†×1300.

PLATE IV

FIGS. 34–46. Chromosome II short arm.

FIG. 34. *IIS-4* (*P. hirtipes*).*
FIG. 35. Sections 52–57 (*P. mixtum*).*
FIG. 36. Sections 48*c*–49 (*P. hirtipes*).‡
FIG. 37. Expanded centromere region (*P. vigintiquaterni*).†
FIG. 38. *IIS-1* (*P. fontanum*).†
FIG. 39. IIS-2 (*P. fontanum*).†
FIG. 40. Sections 48–53 (*P. mixtum*).*
FIG. 41. Sections 45–50 (*P. mixtum*).*
FIG. 42. IIS-5 (*P. vigintiquaterni*).†
FIG. 43. *IIS-6.7* (*P. inflatum*).†
FIG. 44. *IIS-6.7.8.9* (*P. inflatum* aff. 3).†
FIG. 45. IIS-6.7/IIS-6.7.8.9 (*P. inflatum* aff. 3).†
FIG. 46. *IIS-3* and *IIS-4* (*P. hirtipes*).‡

ABBREVIATIONS: RB, Ring of Balbiani; Db, "double bulge".
*×2500.
†×1400.
‡×1050.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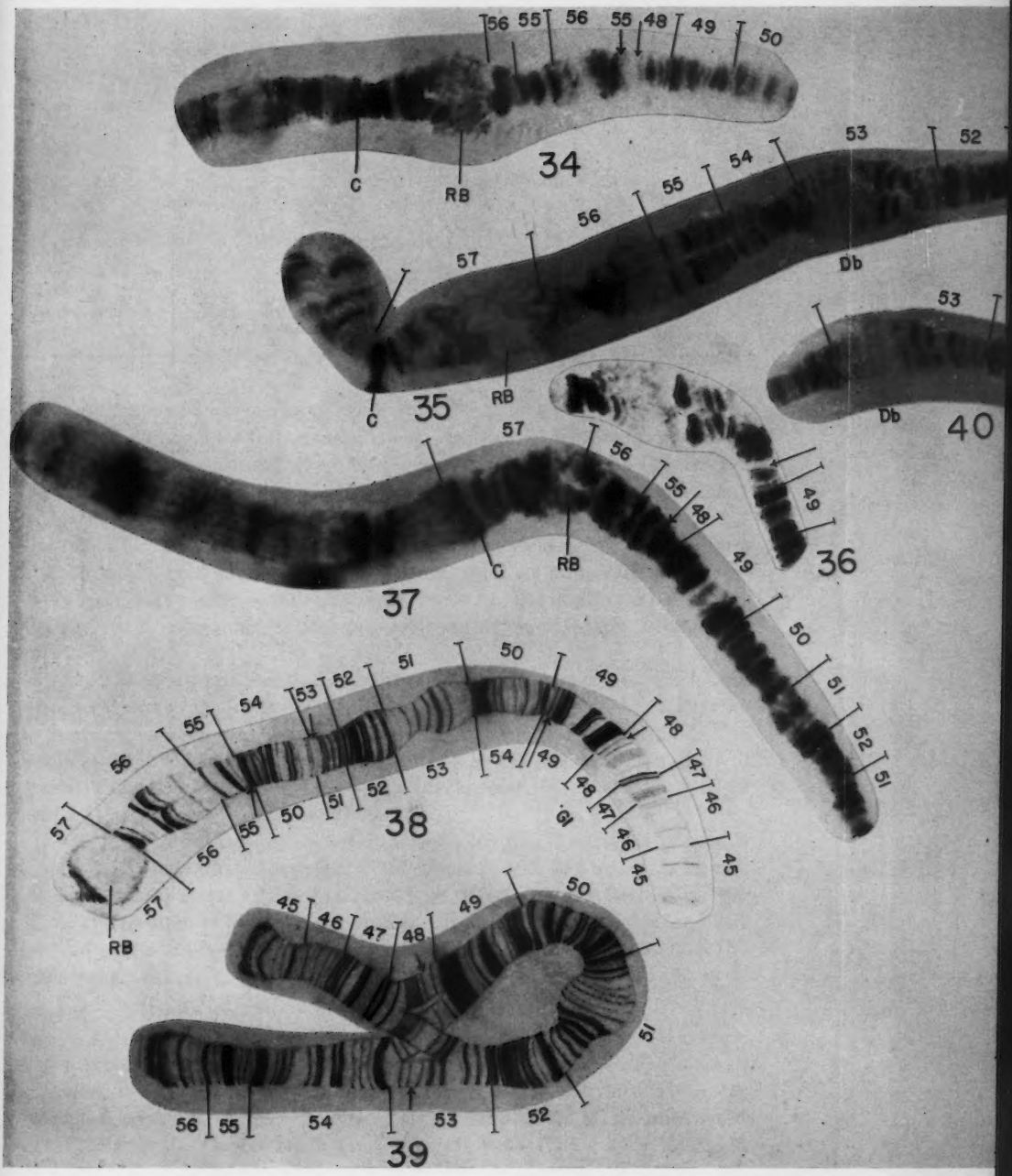
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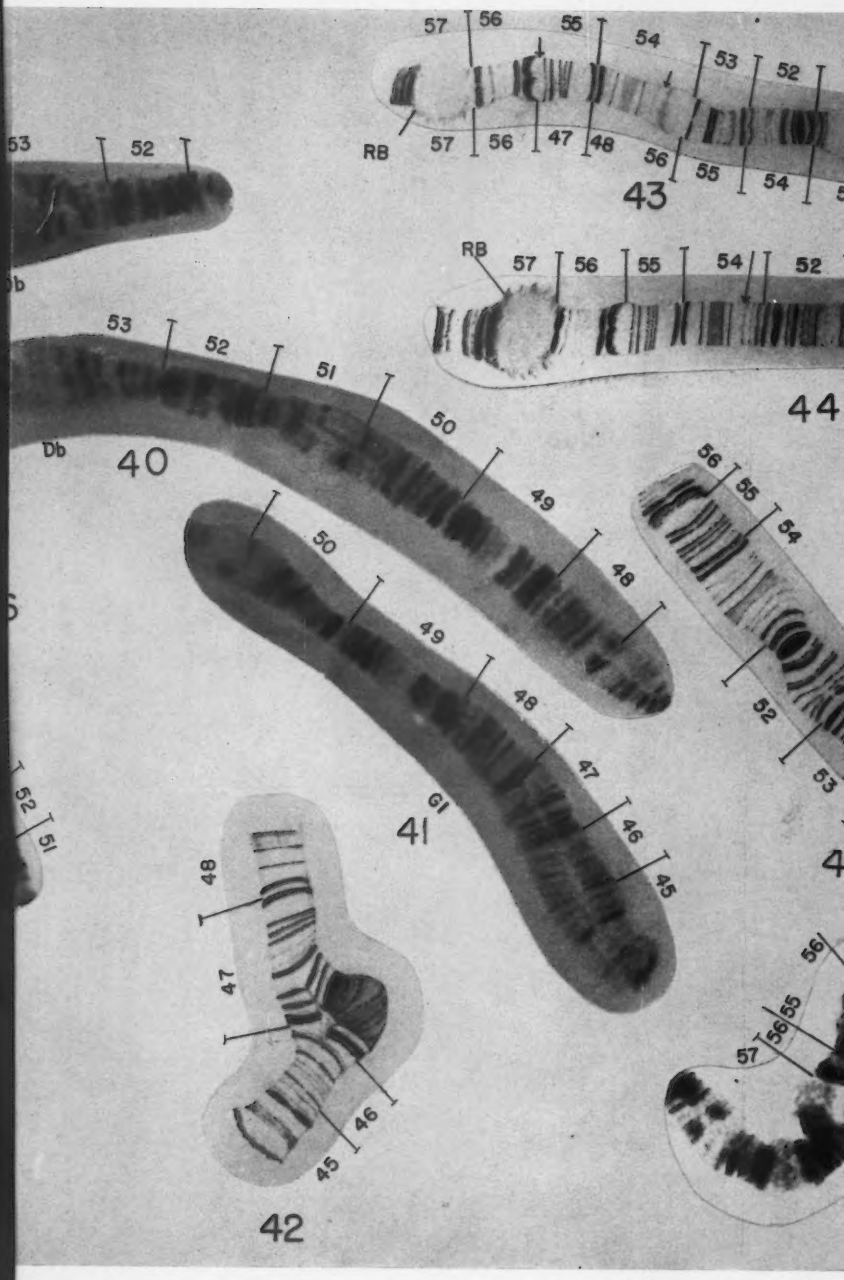
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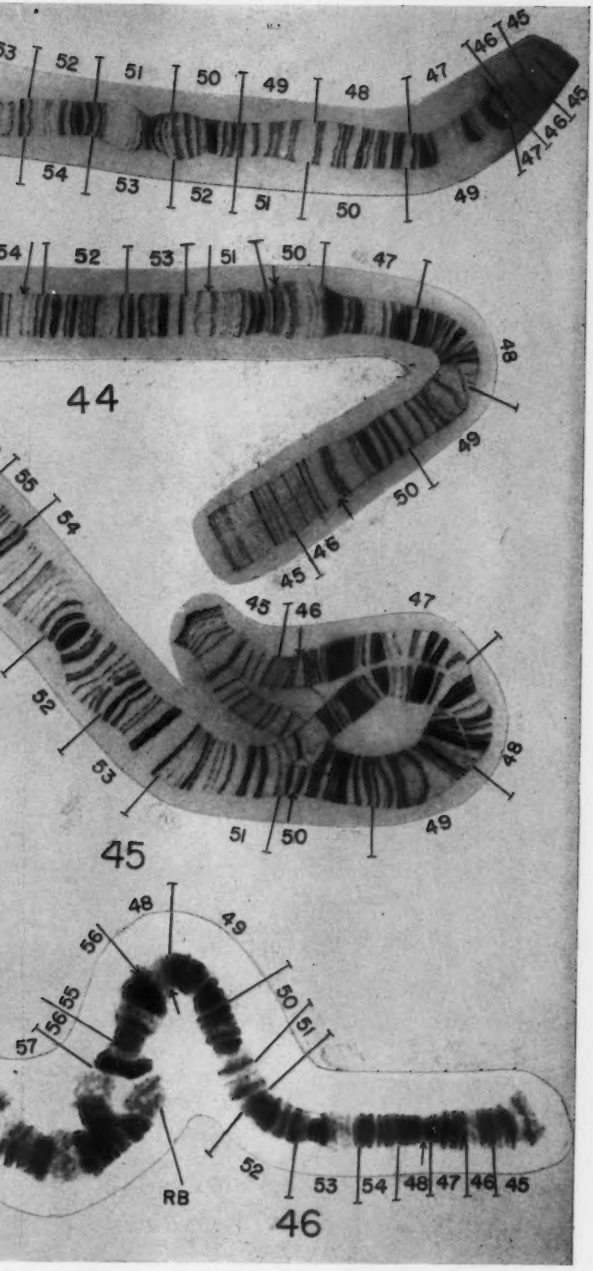
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TABLE VI

Description of inversions on the long arm of chromosome I

P. fontanum	
IL-1	20′–20′*c*.21′*c*–20′*c*.21′*c*–44
P. hirtipes	
IL-2	20′–21′*d*.29*c*–21′*d*.29*c*–44
IL-3.4	20–21*d*.30/29–28*p*.24/25–28*p*.25/24–21*d*.29/30–44
IL-5	20′–24/25.27*c*–25/24.27*c*–44
P. inflatum	
IL-6	20′–21′*c*.23*c*–21*c*.23*c*–44
P. inflatum aff. 1	
IL-7	20′–39*c*.42*c*–39*c*.42*c*–44
P. inflatum aff. 2	
IL-8	20′–26*c*.28*c*–26*c*.28*c*–44

The simultaneous occurrence of IIIL-4 and IIIL-2 in a single larva set us to look for the significance in the association. Out of 181 larvae observed only one showed this association, which seems to suggest that it is by chance that IIIL-2 and IIIL-4 are in the same individual. IIIL-4 also bears no relation to the cytological sex-determining mechanism.

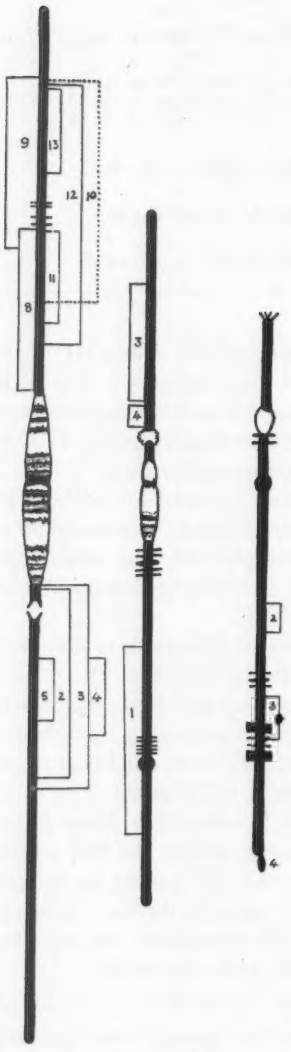
Larvae of *P. hirtipes* from Scotland are entirely identical with those from England in the predominant banding pattern of all six arms. However, the two populations have entirely different sets of floating inversions. No floating inversion common to both populations was found in any chromosome arm.

IS of *P. hirtipes* from Scotland bears three floating inversions of which only one, IS-11, extending from 10*p*–15*c* (Fig. 23), is common. Table III shows the frequencies of homozygotes and heterozygotes in this population. The two floating inversions, IS-12 extending from 3*c*–15/16 (Fig. 22), and IS-13 extending from 3*d*–7*p* (Fig. 21), have one break point, 3*d*, coincident. IS-12 and IS-13 appeared only once in 26 larvae.

IL of *P. hirtipes* from Scotland has three floating inversions of which IL-2 (Fig. 28), extending from section 21*d*–29*c*, was seen in 8 out of 26 larvae. The homozygous sequence for IL-2 could be detected easily because of the shift in the position of the nucleolar region. In one larva a complex loop was observed on IL in which one constituent was standard and the other had the gene arrangement (Fig. 29) as shown below:

20–21*d*.30/29–28*p*.24/25–28*p*.25/24–21*d*.29/30–44.

This gene arrangement can be derived from standard in two steps: one step, IL-3, inverting section 29/30–21*d* and another step, IL-4, reinverting a small section 24/25–28*p*, within the first inverted segment. IL-3.4 thus constitutes a complex inversion of the "included" type. IL-5 (Fig. 27) extends from 24/25–27*c* and shares one break point (24/25) with IL-4. This inversion was seen twice in 26 larvae.



Text-fig. 3. *Prosimulium hirtipes.*

No common floating inversions were detected on chromosome II and chromosome III. However, in one individual an inversion (IIL-2), extending from 58*c*–60*c*, was seen heterozygously (Fig. 48).

The cytological characteristics of *P. hirtipes* are summed up in Text-fig. 3. The floating inversions in populations from England and Scotland are shown together by brackets on the right side of each chromosome arm.

Prosimulium vigintiquaterni Enderlein

Cytological investigations were carried out on several batches of larvae from various streams in Czechoslovakia (Table I). The salivary gland nuclei of *P. vigintiquaterni* have no chromocenter. The three giant chromosomes are generally well stretched. The expanded region of chromosome I is as described previously for *P. fontanum* and *P. hirtipes*. The expanded region of chromosome II is generally more elaborate in *P. vigintiquaterni* than in *P. fontanum* or *P. hirtipes* (Fig. 37).

IS of *P. vigintiquaterni* is involved in the cytological sex-determining mechanism. The inversions which differentiate its constituents into *X* and *Y* are discussed under the section for floating inversions.

IIS of *P. vigintiquaterni* shares the interspecific inversion *IIS-3* with *P. hirtipes* (Figs. 36 and 37). *IIS-4* of *P. hirtipes* is missing in *P. vigintiquaterni*.

Another interspecific rearrangement which *P. vigintiquaterni* shares with *P. hirtipes* is *IIL-1*, which inverts one-third of the length of the IIL arm (Figs. 54, 57).

No interspecific rearrangement was detected on chromosome III.

P. vigintiquaterni has two inverted regions in IS in all females. One inversion, IS-14, brings the "3 heavy groups" in apposition with the four prominent bands in section 11. The break points of inversion IS-14, shown by arrows in Fig. 13, fall in sections 12*d* and 18*p*. IS-15 is a small inversion involving just five bands in section 18′ (18′*p*–18′*c*). In all males, IS forms a major loop in segment 12*d*–18*p* while the segment 18′*p*–18′*c* remains unpaired. In addition, in males, there is a small inverted region (IS-16) confined to two bands, the dense double band of the "3 heavy groups". In the larval samples we examined, no male was seen without these three heterozygous inversions and all females were homozygous for inversions IS-14 and IS-15. From these observations we infer that the cytological sex dimorphism of *P. vigintiquaterni* resides in IS, that inversions IS-14 and IS-15 are probably independent steps in the differentiation of *X*, and that inversion IS-16 is associated with *Y*. The band sequence of *X* and *Y* are given below:

X: 1–12*d*.18′*p*–12*d*.18′*c*–18′*p*.18′*c*–19′,
Y: 1–16/17.17*p*–17/16.17*p*–19′.

In one larva a floating inversion (IS-17) which overlaps inversion IS-14 on the *X* chromosome was seen. The break points of IS-14.17 (17*p* and II*d*) are shown in Fig. 12.

No floating inversion was seen on IIS; but a non-conformity, IIS-5 (Table VII), was detected in three larvae in which a heterochromatic piece is present

in one constituent (Fig. 42). No bands are inverted nor is any band missing in either constituent, which indicates that the heterochromatic piece is an extra segment in the chromosome.

A common inversion IIL-3 occurs heterozygously (Fig. 55) in 19 out of 50 larvae. The break points of this inversion, 64*c* and 70*d* (Table VIII) fall within the limits of the interspecific inversion *IIL-1*. The gene sequence of IIL-3 is as shown below:

58–64*p*.71/70–70*d*.64*c*–70*d*.64*c*–64*p*.70/71–74.

Table III shows the frequencies of heterozygotes and homozygotes for IIL-3.

TABLE VII

Descriptions of inversions on the short arm of chromosome II

P. fontanum	
IIS-1	45–49/50.55*c*–50/49.55*c*–57
IIS-2	45–48*d*.51*p*–55*c*.49/50–48*d*.51*p*–50/49.55*c*–57 (45–48*c*.53*d*–48*c*.53*d*–57)
P. hirtipes and *P. vigintiquaterni*	
IIS-3	45–48*c*.55*c*–48*c*.55*c*–57
P. hirtipes	
IIS-4	45–55*c*.56*p*–55*c*.56*p*–57
P. vigintiquaterni	
IIS-5	45–47*p*.heterochromatin.47*p*–57
P. inflatum	
IIS-6.7	45–47*p*.48*d*–56*c*.48*d*–47*p*.56*c*–57
P. inflatum aff. 3	
IIS-8	45–47*p*.48*d*–53*d*.56*c*–53*d*.48*d*–47*p*.56*c*–57
IIS-8.9	45–47*p*.52*d*–48*d*.52*d*–53*c*.56*c*–53*c*.48*d*–47*p*.56*c*–57 (45–47*p*.50*d*–47*p*.50*d*–51*d*.54*p*–51*d*.54*p*–57)

TABLE VIII

Descriptions of inversions on the long arm of chromosome II

P. hirtipes and *P. vigintiquaterni*	
IIL-1	58–64*p*.71/70–64*p*.70/71–74
P. hirtipes	
IIL-2	58–58*c*.60*c*–58*c*.60*c*–74
P. vigintiquaterni	
IIL-1.3	58–64*p*.71/70–70*d*.64*c*–70*d*.64*c*–64*p*.70/71–74
IIL-1.4	58–64*p*.71/70–64*c*.73/72–70/71.64*p*–64*c*.72/73–74
IIL-1.5.6.7.8	58–64*p*.71/70–70*d*.67*p*–65*p*.67*p*–70*d*.64*c*–64*p*.70/71–72*c*.64*c*–65*p*.72*c*–74
P. inflatum group	
IIL-9	58–59*p*.62/61–59*p*.61/62–74
IIL-9.10	58–59*p*.62/61–59*p*.69/68–61/62.68/69–74
P. inflatum aff. 2	
IIL-9.10.11	58–59*p*.62/61–59*p*.69/68–61/62.68/69–69*c*.73/72–69*c*.72/73–74

A complex loop (Fig. 56) was seen on IIL in one larva in which both constituents differ from the species (*P. vigintiquaterni*) standard IIL sequence. One constituent differs from the species standard in a simple inversion IIL-4 which is superimposed on the interspecific rearrangement *IIL-1*. IIL-4 shifts the heavy band 64*p* of the "capsule" to the distal one-third of section 72. The other constituent has many inverted segments which could be written as shown below:

58–64*p*.71/70–70*d*.67*p*–65*p*.67*p*–70*d*.64*c*–64*p*.70/71–72*c*.64*c*–65*p*.72*c*–74.

This sequence could be arrived at through a series of simple inversion steps on the universal standard. The minimum number of inversion steps required to derive this gene arrangement from universal standard is five, of which the first will be the interspecific inversion step *IIL-1*. The probable steps that might have followed are shown below:

IIL-5 inverting the segment 65*p*–72*c*,
IIL-6 inverting the segment 64*c*–72*c*,
IIL-7 inverting the segment 65*p*–70*d*,
IIL-8 inverting the segment 65*p*–67*p*.

An inversion, IIIS-1, involving segment 76*d* to 77*c* was seen heterozygously in four larvae (Fig. 58). This is the only inversion seen on IIIS of the species in this group.

No common floating inversion was detected on IIIL. A complex loop (Fig. 65) was seen in five larvae from the Jeseniky mountain region. One constituent of this loop is standard while in the other, two major inverted segments and a heterochromatic piece were detected. The gene sequence of the rearranged constituent is shown below:

83–84*p*.84*d*–84*p*.heterochromatin.88*c*–84*d*.88/89–100.

The segment which is missing on the inverted constituent is 88*c*–88/89. The heterochromatic block might be a compound of the four bands in standard segment, 88*c*–88/89, or it might include an extra section, in addition to these four bands. The two inverted pieces 84*p*–84*d* and 84*d*–88/89 seem to be the products of two independent inversion steps, IIIL-5 and IIIL-6, respectively.

Another floating inversion constituting a complex loop in the heterozygous condition was seen in two larvae (Fig. 73). The rearranged constituent in the loop has three major inverted segments and a heterochromatic piece. The gene sequence in this constituent could be represented as:

83–90/91.heterochromatin.94*d*–92*d*.97*p*–94*d*.92*d*–92*c*.97*p*–100.

The segment which is missing in this constituent is 90/91–92*c*. The bands which have turned heterochromatic are hard to recognize as they have lost their physical characteristics. If the heterochromatic blocks include segment 90/91–92*c* in its normal orientation, the minimum number of inversion steps required to derive the inverted sequence from standard IIIL will be three: the first step IIIL-7, inverting segment 92*c*–97*p*, a second step IIIL-8, inverting a piece 92*d*–94*d* (included in the first inverted segment), and a third and final step, IIIL-9, inverting the segment 94*d*–97*p*. If the segment 90/91–92*c* is in

TABLE IX

Descriptions of inversions on chromosome III

P. vigintiquaterni	
IIIS	75–76*d*.77*c*–76*d*.77*c*–82
P. fontanum	
IIIL-1	83–87/88.94*p*–88/87.94*p*–100
P. hirtipes	
IIIL-2	83–87*d*.88*d*–87*d*.88*d*–100
IIIL-3	83–93/92.91/92–92/93.heterochromatin.92/91–100
IIIL-4	83—100 + heterochromatin
P. vigintiquaterni	
IIIL-5.6	83–84*p*.84*d*–84*p*.heterochromatin.88*c*–84*d*.88/89–100
IIIL-7.8.9.10	83–90/91.heterochromatin.94*d*–92*d*.97*p*–94*d*.92*d*–92*c*.97*p*–100
IIIL-11	83—100*c*.heterochromatin.100*d*–100*c*.100*d*–end
P. inflatum	
IIIL-12	83–85*p*.87*c*–85*p*.87*c*–100
IIIL-13	83–90*p*.heterochromatin.92/91–90*c*.90/91–100
P. inflatum aff. 3	
IIIL-14.15	83–84*c*.85*d*–84*d*.86*d*–85*d*.84*c*–84*d*.86*d*–100

inverted orientation with respect to standard, one more inversion step IIIL-10 involving 91/90–92*c* will be required. On the strength of the tentative identification of the "triad" and the heavy band in section 91 the latter interpretation is favored.

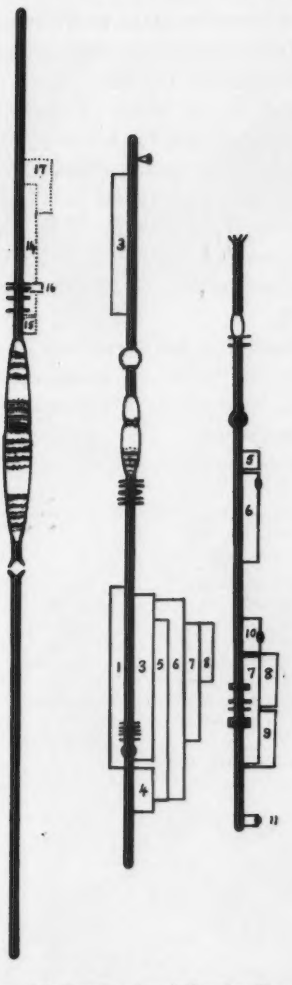
IIIL has another non-conformity IIIL-11, associated with an extra heterochromatic piece (Table IX) at the end of the arm (Fig. 74). Such IIIL arms have a small segment inverted (shown by arrows in Fig. 74) in section 100, in addition to having an extra heterochromatic piece. This condition was observed heterozygously in six larvae.

The larvae of *P. hirtipes arvernense* from Central France were all immature; consequently we could not obtain good cytological preparations from them. Of the 12 larvae sent by D. M. Davies, only five made passable slides. The salivary gland chromosomes, although poor, showed very striking similarity to those of *P. vigintiquaterni* from Czechoslovakia. The predominant sequence in all six arms of *P. hirtipes arvernense* is identical with that of *P. vigintiquaterni*, except for IIL where the rare Czechoslovakian sequence IIL-3 was found in all five slides in place of *IIL-1*, common in Czechoslovakia.

The floating inversion IS-14 of *P. vigintiquaterni* was seen in one of the larvae from Central France. Because of the poor quality of the slide it is difficult to say whether IS-15 and IS-16 were also present. The occurrence of IS-14 in *P. hirtipes arvernense* and the absence of any interspecific inversion differences between the forms from Czechoslovakia and Central France lead us to the conclusion that these two forms belong to one and the same species.

The single Austrian larva is identical in its cytological features with the females of *P. hirtipes arvernense.*

The idiogram (Text-fig. 4) is based on the cytological characteristics of *P. vigintiquaterni* from Czechoslovakia. The % TCL of *P. hirtipes arvernense* of Austria and Central France tally with that of *P. vigintiquaterni.*



Text-fig. 4. *Prosimulium vigintiquaterni* and *Prosimulium hirtipes arvernense.*

Prosimulium inflatum Davies

Two batches of larvae from Scotland (Table I) supplied the cytological information presented here. The salivary gland nuclei of *P. inflatum* display a strong chromocentric tendency, although the presence of a tight chromocenter is not universal in *P. inflatum.*

Excepting the highly modified expanded region, chromosome I of *P. inflatum* in females is standard.

The gene sequence of IIS in *P. inflatum* could be described in terms of standard as follows:

45–47*p*.48*d*–56*c*.48*d*–47*p*.56*c*–57.

Standard IIS requires two inversion steps to arrive at this gene sequence: one step, here referred to as *IIS-6*, inverting a large segment, 47*p*–56*c*, and another step, *IIS-7*, involving the segment 48*d*–56*c*. Since neither of these inversions was seen alone, it is hard to say which of these inversions occurred first. The three arrows in Fig. 43 indicate the break points of *IIS-6.7*. The arrow in section 56*c* indicates the break point shared by *IIS-6* and *IIS-7*. *IIS-7* leaves the majority of the bands in normal orientation while the subterminal segment 47*p*–48*d* is inserted at the base of IIS in reversed sequence.

The section numbers above in Fig. 43 show the species (*P. inflatum*) standard IIS sequence. The numbers below indicate the relation of this rearrangement to the universal standard.

IIL of *P. inflatum* carries a fixed rearrangement, *IIL-9*, inverting the segment 59*p*–61/62 (Fig. 50). This inversion shifts the "group of 5" from the very base of the IIL arm to the neighborhood of section 62 in the *P. inflatum* standard gene sequence.

No interspecific inversion was seen on chromosome III.

PLATE V

FIGS. 47–57. Chromosome II long arm.

FIG. 47. Sections 58–65 (*P. mixtum*).*
FIG. 48. IIL-2/IIL (*P. hirtipes*).†
FIG. 49. *IIL-11* (*P. inflatum* aff. 2).‡
FIG. 50. *IIL-9*.10 (*P. inflatum*).‡
FIG. 51. *IIL-9/IIL-9*.10 (*P. inflatum*).†
FIG. 52. Sections 64–71 (*P. mixtum*).*
FIG. 53. Sections 70–74 (*P. mixtum*).*
FIG. 54. Sections 63–74 (*P. vigintiquaterni*).‡
FIG. 55. IIL-1/IIL-3 (*P. vigintiquaterni*).†
FIG. 56. IIL-1.4/IIL-5.6.7.8 (*P. vigintiquaterni*).†
FIG. 57. Entire IIL (*IIL-1*) (*P. hirtipes*).‡

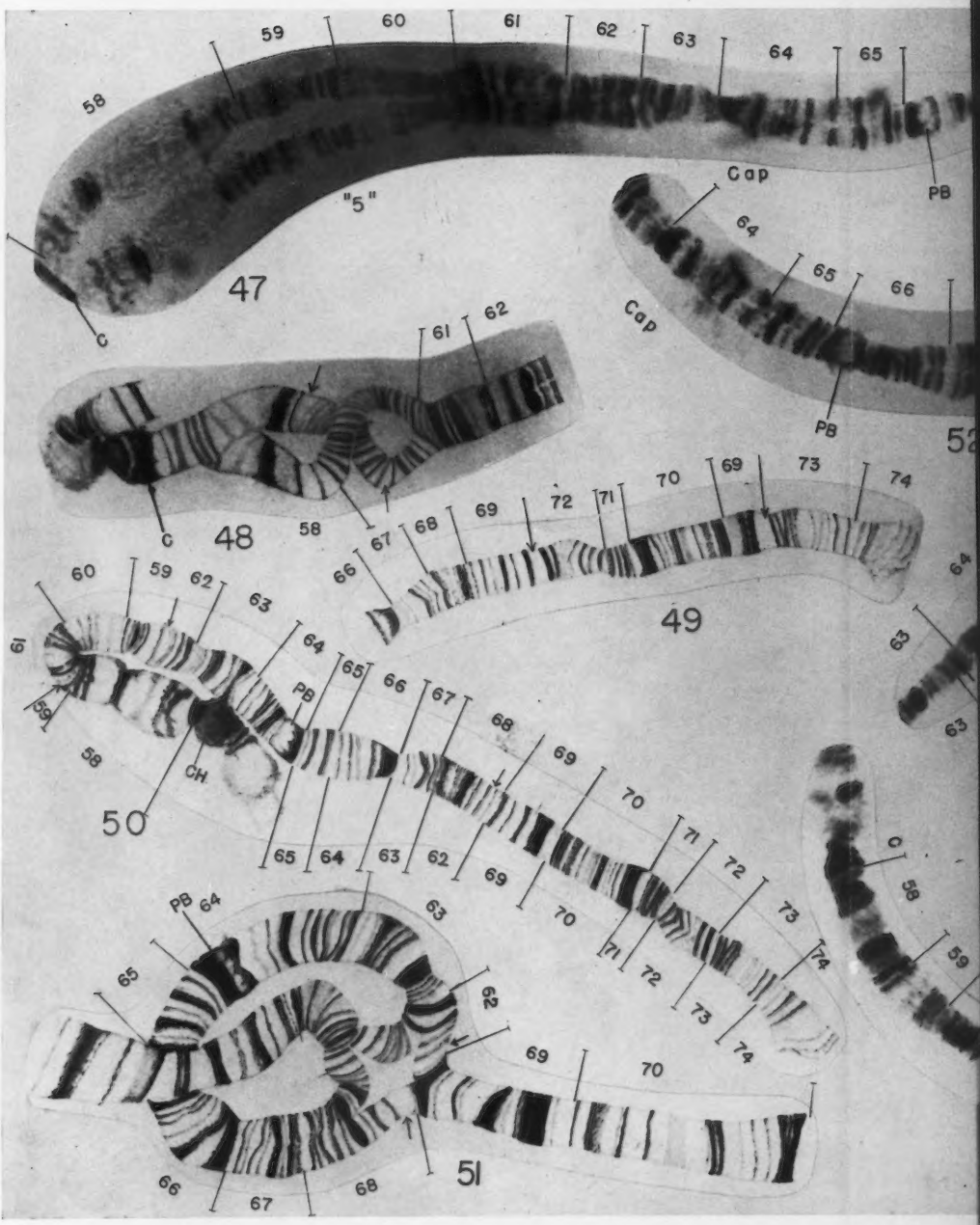
ABBREVIATIONS: PB, para Balbiani; Cap, capsule; "5", the "group of 5".
*×2300.
†×1300.
‡×1000.

PLATE VI

FIGS. 58–76. Chromosome III.

FIG. 58. IIIS Sections 75–77 (*P. vigintiquaterni*).*
FIG. 59. IIIS Sections 75–82 (*P. mixtum*).*
FIG. 60. IIIL/IIIL-2 (*P. hirtipes*).†
FIG. 61. IIIL Sections 86–92 (*P. fuscum*).*
FIG. 62. IIIL Sections 89–94 (*P. fuscum*).*
FIG. 63. IIIL Sections 93–100 (*P. fuscum*).†
FIG. 64. IIIL Sections 83–93 (*P. mixtum*).*
FIG. 65. IIIL/IIIL-5.6 (*P. vigintiquaterni*).†
FIG. 66. IIIL/IIIL-14.15 (*P. inflatum* aff. 3).†
FIG. 67. IIIL/IIIL-12 (*P. inflatum*).†
FIG. 68. IIIL Sections 90–93 (*P. mixtum*).*
FIG. 69. *IIIL-1* (*P. fontanum*).‡
FIG. 70. IIIL-13 (*P. inflatum*).†
FIG. 71. IIIL Sections 88–96 (*P. hirtipes*).‡
FIG. 72. IIIL Sections 91–100 (*P. mixtum*).*
FIG. 73. IIIL/IIIL-7.8.9.10 (*P. vigintiquaterni*).†
FIG. 74. IIIL/IIIL-11 (*P. vigintiquaterni*).†
FIG. 75. IIIL/IIIL-3 (*P. hirtipes*).*
FIG. 76. IIIL/IIIL-4 (*P. hirtipes*).*

ABBREVIATIONS: F, frazzle; Bl, blister; ST, "shield and triad".
*×2900.
†×1600.
‡×1200.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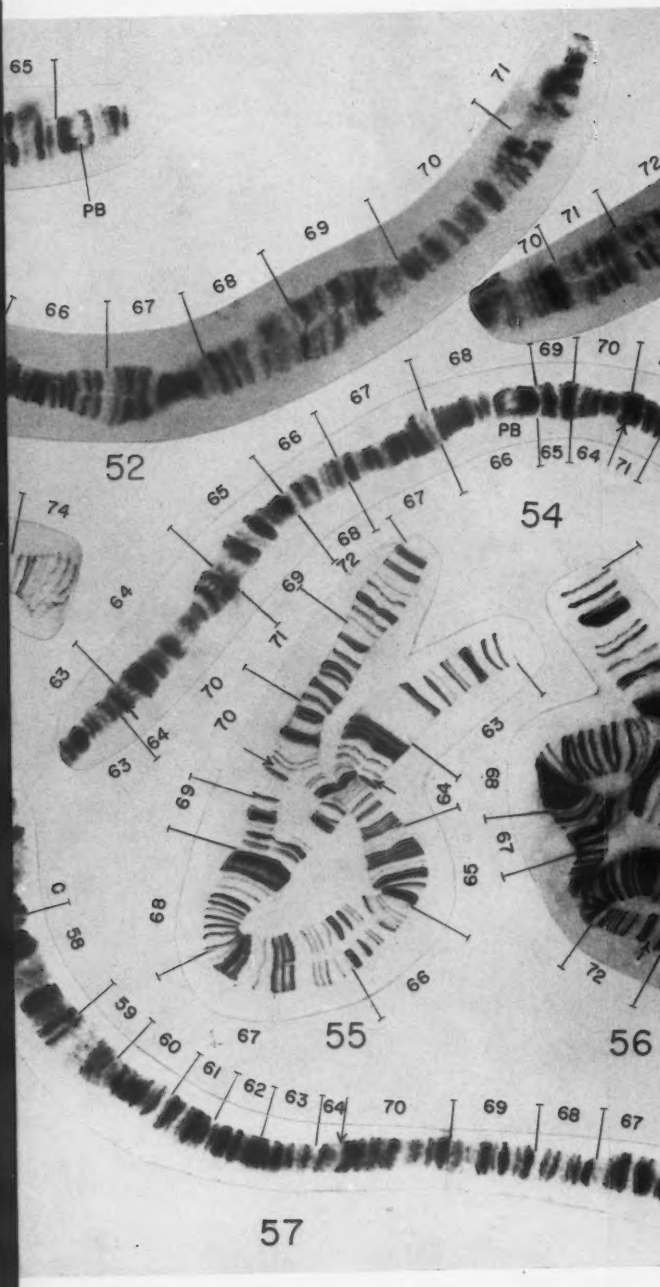
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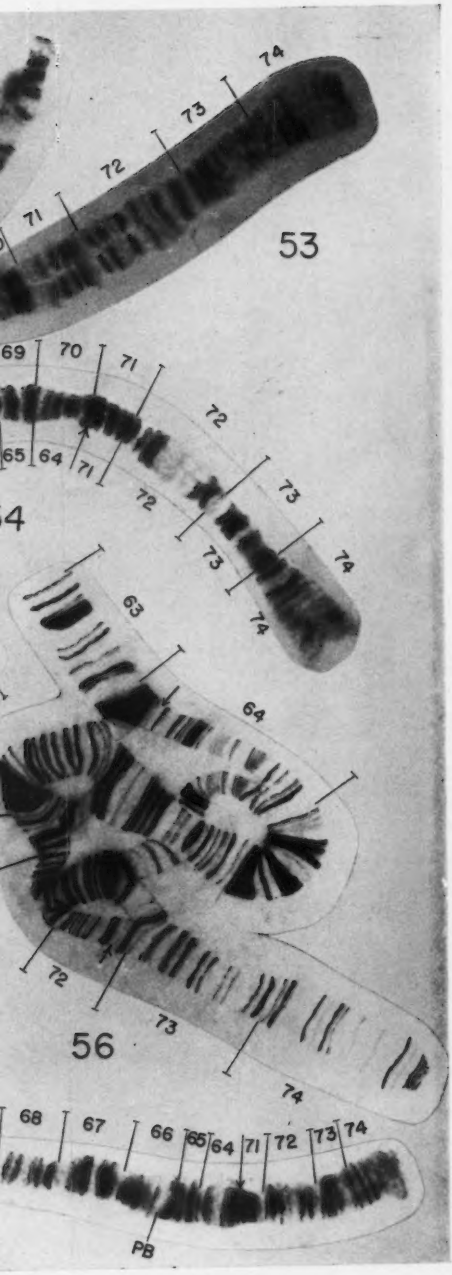
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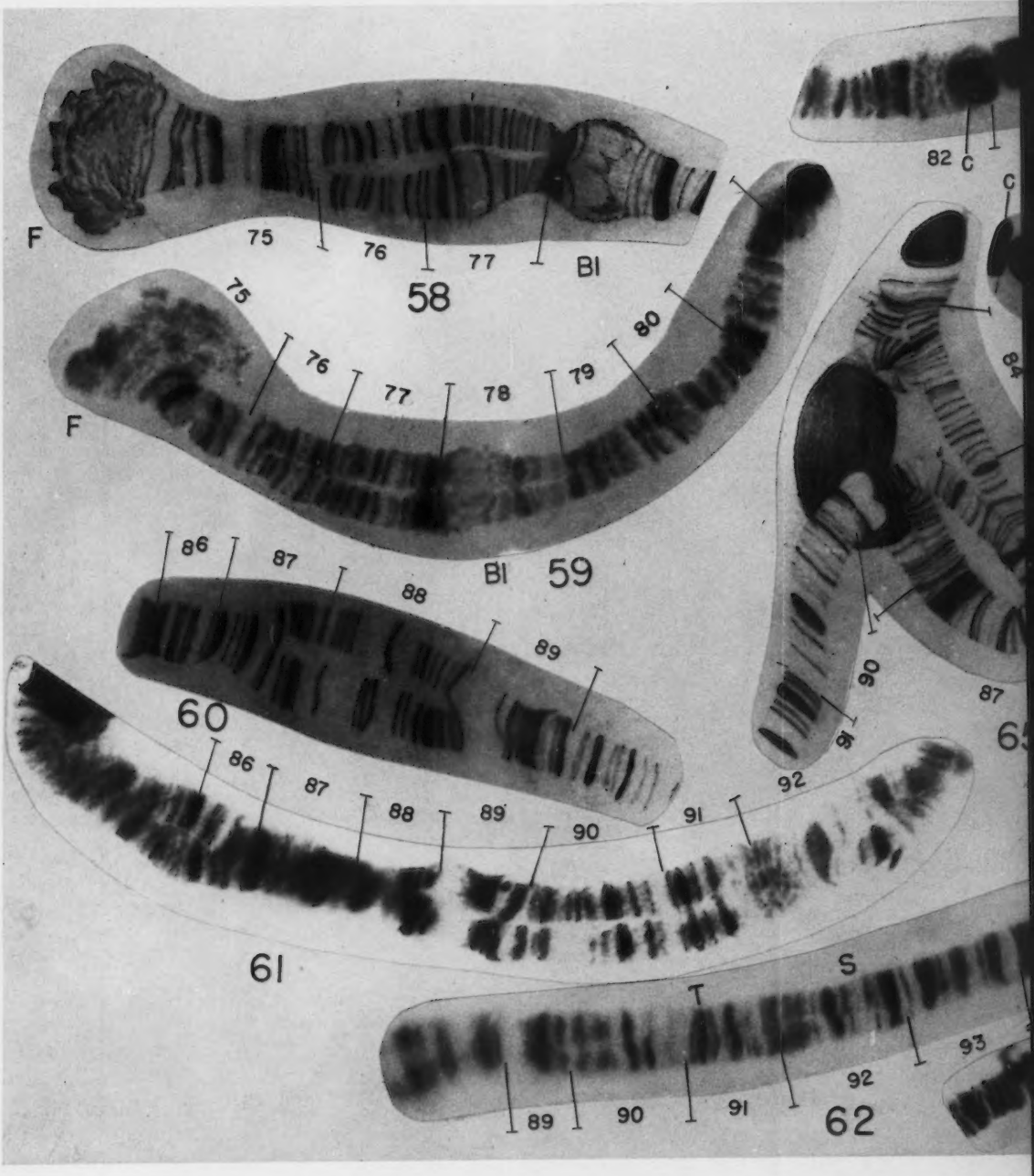
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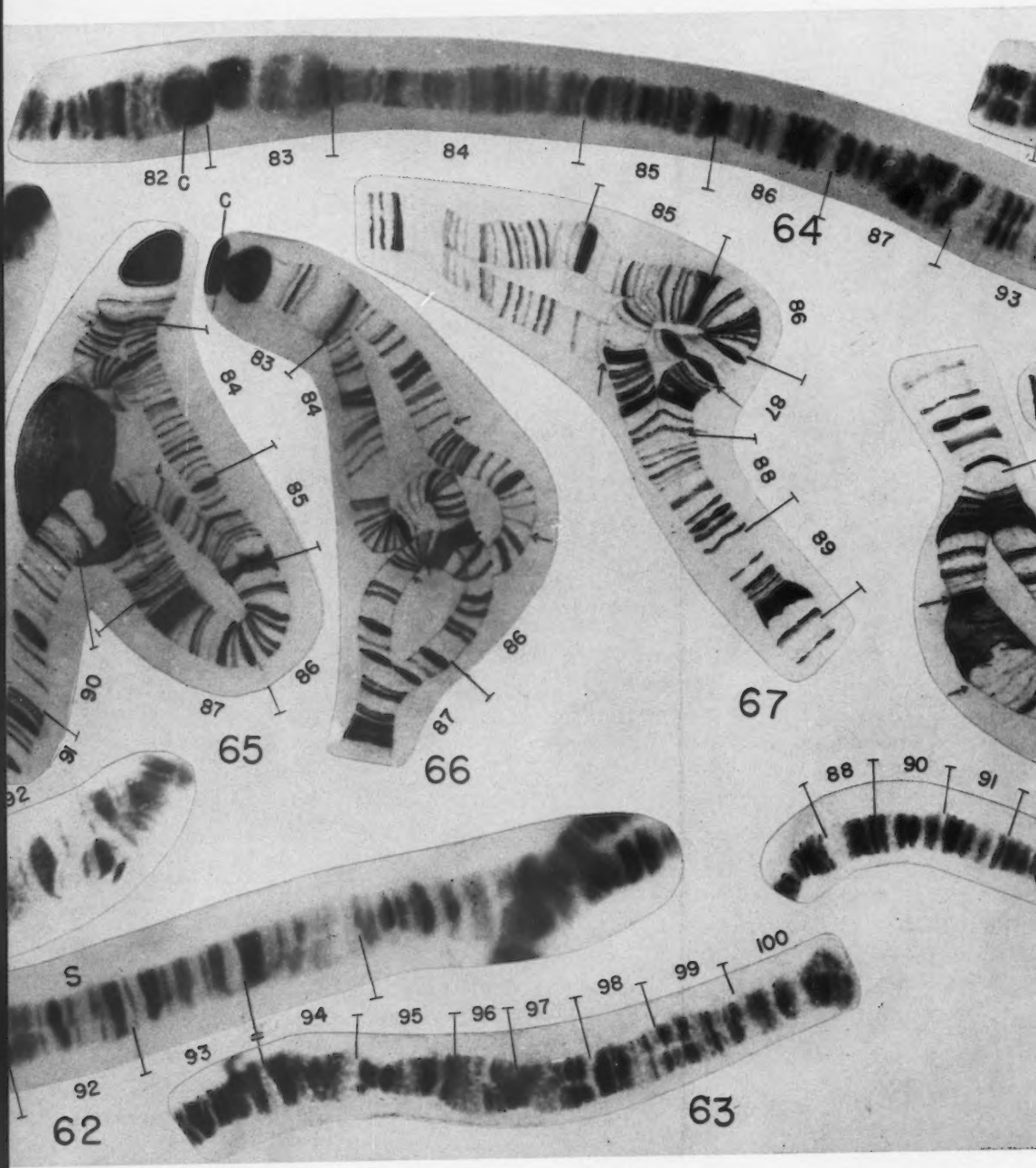
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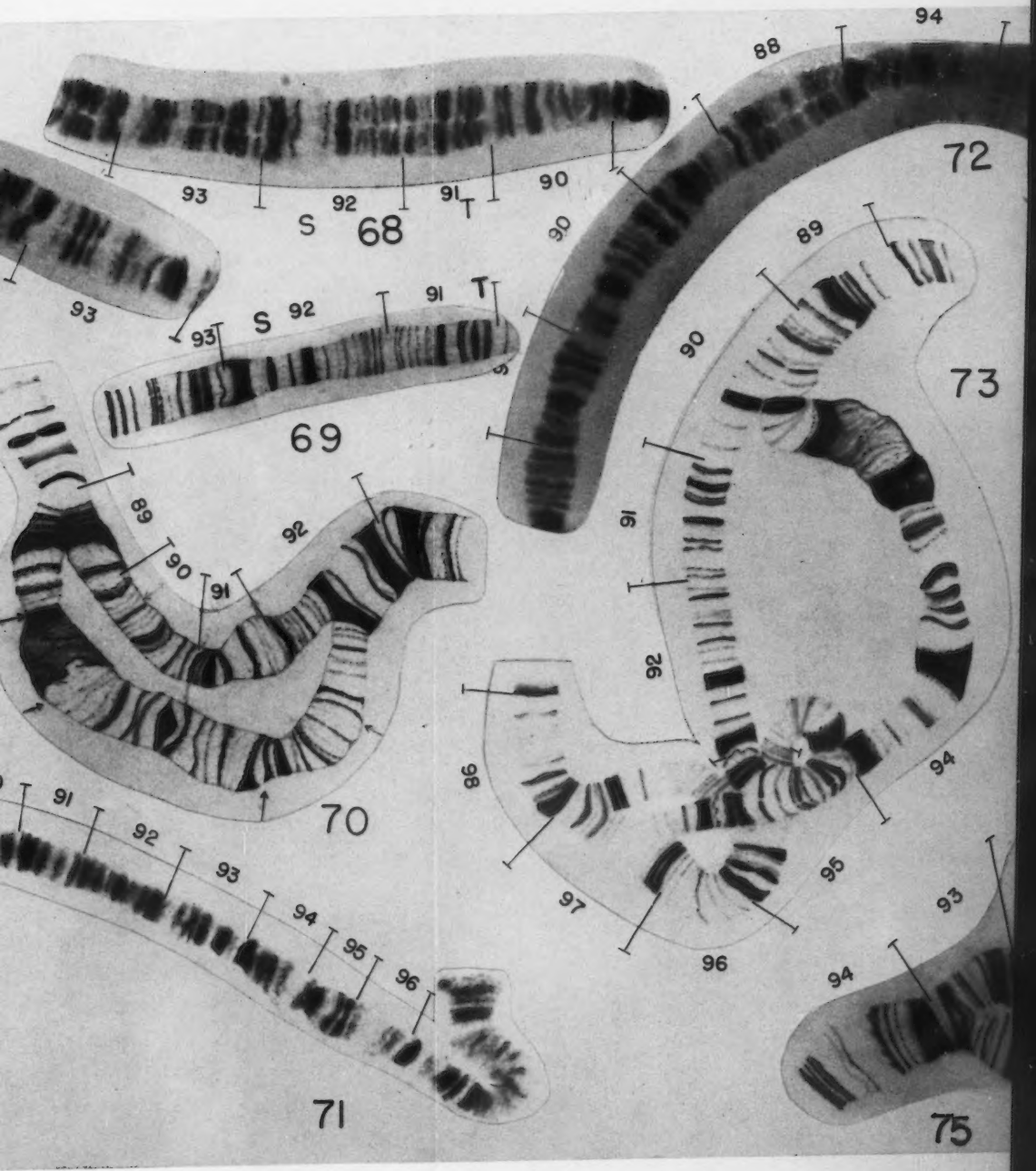
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A rearrangement resulting in non-pairing of the centromere region of chromosome I was seen in 17 larvae, 15 of which were males. The remaining two larvae could not be sexed as they were immature. The four heterochromatic bands in section 20′ appeared to be reversely paired in some nuclei; but because of the inconsistency in their expression it is difficult to give a definitive interpretation of the rearrangement involved. In addition, in all males, an inversion (IL-6) involving segment *21′c–23d* (Fig. 25) was also detected on one constituent.

The rearrangement in the centromere region and the inversion IL-6 were always found together in all males. The absence of these rearrangements in females and the presence of this heterozygosity in all males suggest that IL-6 and the rearrangement at the centromere region are associated with the sex-determining mechanism and that these changes are on the *Y* chromosome. In males chromosome I was often broken at the centromere region. Where it was not broken, the nucleolar gap was seen only on one constituent, presumably on the *X* (Fig. 25).

A floating inversion IIL-10, involving segment 61/62–68/69 (Fig. 51) was seen in great frequency in *P. inflatum*. In all but two larvae this inversion was seen in homozygous condition while the standard IIL sequence was seen only in two larvae which were heterozygous for inversion IIL-10. Thus the predominant IIL sequence of *P. inflatum* has both *IIL-9* and IIL-10 (Fig. 50) and could be represented as follows:

58–59*p*.62/61–59*p*.69/68–61/62.68/69–74.

The break point, 62/61, is shared by *IIL-9* and IIL-10.

Two floating inversions IIIL-12 and IIIL-13 were found in *P. inflatum*. The former extends from *85p–87c* (Fig. 67) and was seen in 3 out of 28 larvae. The arrangement IIIL-13 was detected twice in the population and had the gene sequence (Fig. 70) shown below:

89–90*p*.heterochromatin.92/91–90*c*.90/91–100.

The four light bands of segment *90p* to *90c* are missing and the segment 90/91–91/92 is repeated. The order of genes in IIIL-13 shows that it is a reversed repeat (Fig. 70).

Prosimulium larvae sent by D. M. Davies from various collection sites in Europe could be placed in two cytological categories: the six samples from the Lac d'Oredon, Haute (High) Pyrenees, France, and one sample from St. Moritz, Engadin Valley, Swiss Alps, belong to one group while the three samples from the French Alps (with the exception of a single larva) and three samples from Central Massive, France, fall into another group. Since both groups show very striking resemblance with *P. inflatum* from Scotland, we refer to the former (including the one exception from the Grenoble region) as *P. inflatum* aff. 1 and to the latter as *P. inflatum* aff. 2.

Prosimulium inflatum aff. 1

Several samples of this form from the High Pyrenees and the Swiss Alps were cytologically analyzed. Most larvae could not be sexed since they were immature. A weak chromocenter is present in many nuclei in *P. inflatum* aff. 1.

All six chromosome arms of *P. inflatum* aff. 1 have band sequence found also in *P. inflatum* from Scotland: the complex gene sequence on IIS (*IIS-6.7*) and the inversion *IIL-9* of *P. inflatum* are shared by this form.

An inversion, IS-18, was seen heterozygously in 26 out of 44 larvae. One break point of IS-18 falls in 14*p*, close to the "cup and saucer" (26), while the other cuts across the light gap in section 8*p* (Fig. 16).

A rare inversion, IL-7, extending from 39*c*–42*c* (Table VI) was seen in one larva. The break points of IL-7 are shown on Fig. 31.

Another common floating inversion in the High Pyrenees larvae is IIL-10 which was observed also in *P. inflatum* from Scotland. The single exception from Central France had the rearrangement, IIIL-13, already mentioned in connection with *P. inflatum* from Scotland. The presence of these floating inversions together with the fixed inversions *IIS-6.7* and *IIL-9* in *P. inflatum* aff. 1 lead us to the conclusion that this form is *P. inflatum* itself or at least a close relative of *P. inflatum* from Scotland.

No sex-indicative inversion was detected in the 44 larvae examined.

The idiogram (Text-fig. 5) represents the cytological features of both *P. inflatum* and *P. inflatum* aff. 1.

Prosimulium inflatum aff. 2

The larval samples from the Grenoble region and Central France (Table I) provided a few "workable" slides of this form. Like *P. inflatum* aff. 1 these larvae also show striking cytological resemblance to *P. inflatum*. A chromocenter is present in most nuclei, and the centromere regions of chromosomes I and II are like those of other European species discussed before.

IS of this form differs from standard in being always homozygous for *IS-18*, which was seen heterozygously in *P. inflatum* aff. 1 from the High Pyrenees. In other words, *IS-18*, found floating in the High Pyrenees population, has become fixed in *P. inflatum* aff. 2 (at least within the limits of the small sample of 40 larvae).

An inversion, *IL-8*, extending from 26*c*–28*c* (Fig. 33) was seen in homozygous condition in all larvae. This inversion was not seen heterozygously or homozygously in *P. inflatum* or *P. inflatum* aff. 1.

IIS of *P. inflatum* aff. 2 has the complex inversion *IIS-6.7*, already mentioned in connection with *P. inflatum* from Scotland and *P. inflatum* aff. 1 from the High Pyrenees.

IIL of this species differs from standard in three independent inversions two of which, *IIL-9* and *IIL-10*, are present in *P. inflatum* from Scotland and in *P. inflatum* aff. 1, from the High Pyrenees. The third inversion,

Text-fig. 5. *Prosimulium inflatum* and *Prosimulium inflatum* aff. 1.

IIL-11, extends from 69*c* to 72/73 (Fig. 49). This inversion also was not seen heterozygously in the Alpine or Central France population.

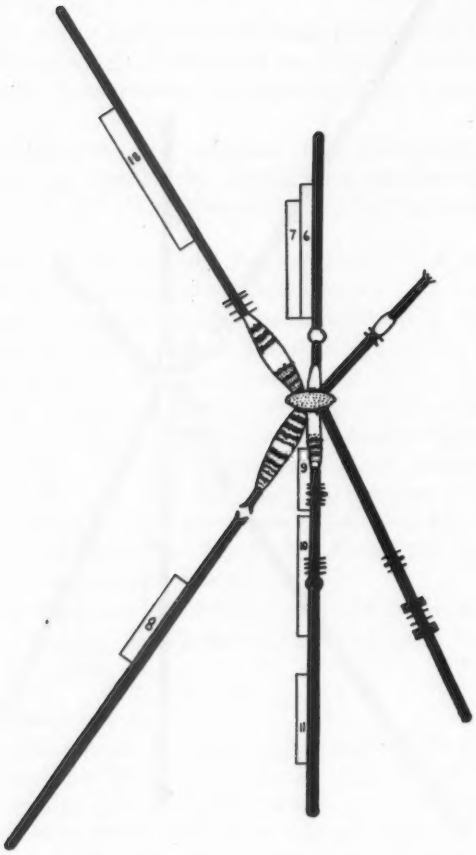
Chromosome III of this species is standard.

No floating inversion, sex-bound or autosomal, was detected in the sample populations we have examined.

Text-fig. 6 shows the cytological features of *P. inflatum* aff. 2.

Prosimulium inflatum aff. 3

Our information on this form comes from the 12 slides prepared 6 years ago and sent to us by E. Kunze-Mühl from Austria. Although the chromosomes in these slides were faintly stained, reliable cytological information could still be derived from them. The salivary gland chromosomes of this form show great similarity to those of *P. inflatum* from Scotland. Some nuclei exhibit a chromocentric tendency.



Text-fig. 6. *Prosimulium inflatum* aff. 2.

IS is standard in *P. inflatum* aff. 3 in that the universal standard sequence for this arm has been observed. No inversion was seen on IL.

There are four well-identified rearranged segments in the predominant IIS sequence (Fig. 44) which could be represented as follows:

45–47*p*.52*d*–48*d*.52*d*–53*c*.56*c*–53*c*.48*d*–47*p*.56*c*–57.

This gene sequence could be arrived at in two more independent inversion steps on *P. inflatum* IIS (Table VIII): one step, *IIS-8*, inverting 53*c*–56*c* and another step, IIS-9, inverting 48*d*–52*d*. *IIS-8* was always seen in homozygous condition but IIS-9 was seen heterozygously in one slide (Fig. 45). This brings the difference between the IIS arms of *P. inflatum* aff. 3 and *P. inflatum* from Scotland to a single inversion step, *IIS-8*.

The common floating inversion in *P. inflatum* aff. 3 is IS-18, which has already been mentioned in connection with the IS arms of the Alpine and the High Pyrenees forms. IS-18 was seen in 5 out of 12 slides we examined, and has been reported to have a 40% frequency (18). A rare floating inversion, IS-19, extending from 16*p* to 17/18, was seen in one slide (Fig. 11).

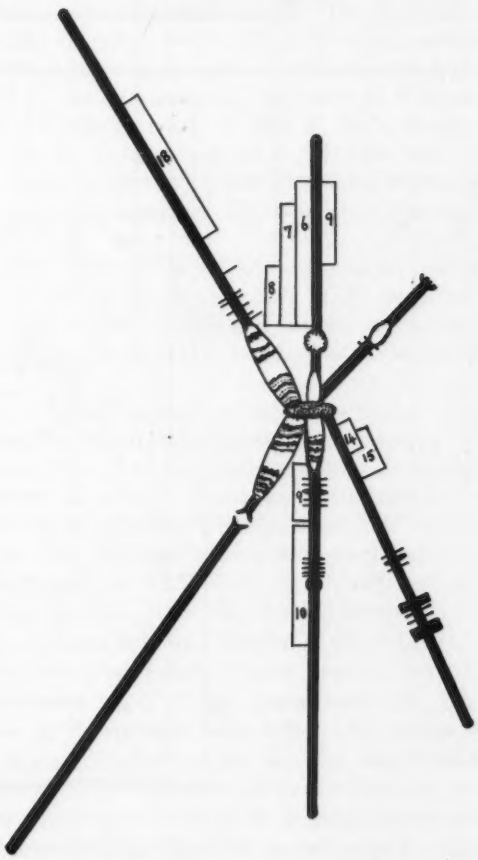
The floating inversion IIS-9 has already been mentioned.

Another rare floating inversion was seen on IIIL which carries three rearranged pieces (Fig. 66) in the gene sequence shown below:

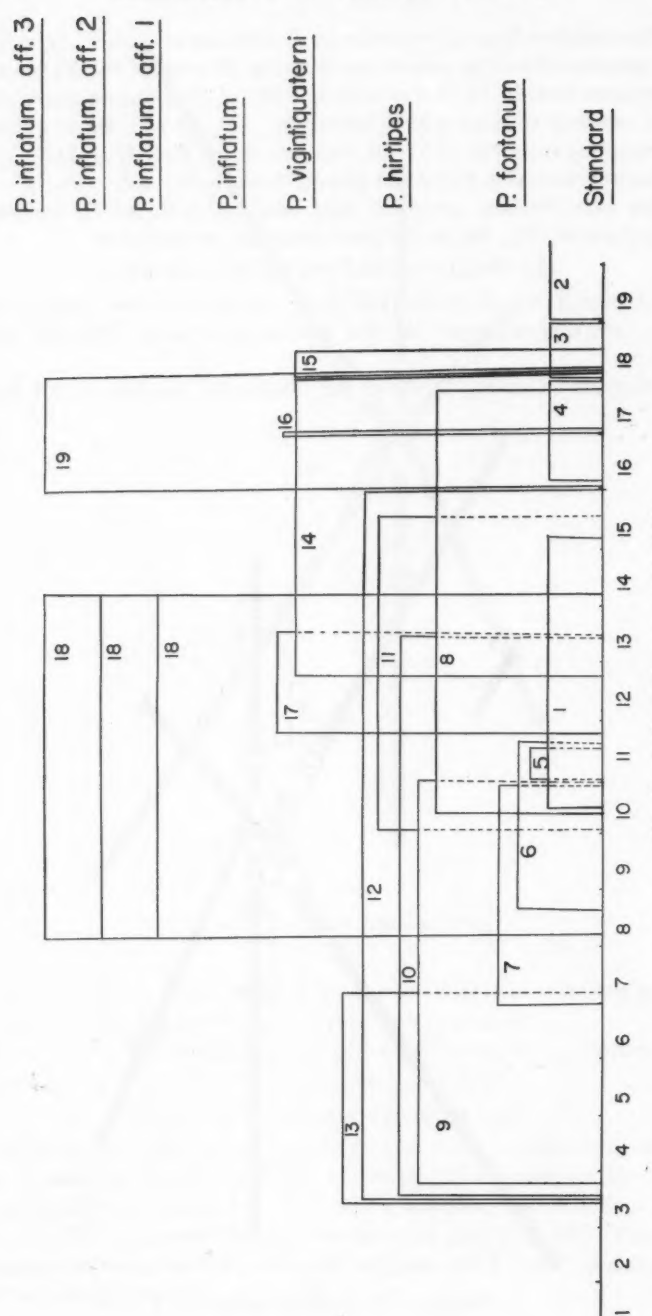
83–84*c*.85*d*–84*d*.86*d*–85*d*.84*c*–84*d*.86*d*–100.

This arrangement could be derived from standard in two steps: one step, IIIL-14, inverting segment 84*c*–85*d* and another step, IIIL-15, inverting 84*d*–86*d*.

The idiogram (Text-fig. 7) shows the cytological features of this form.



Text-fig. 7. *Prosimulium inflatum* aff. 3.


P. inflatum aff. 3
P. inflatum aff. 2
P. inflatum aff. 1
P. inflatum
P. vigintiquaterni
P. hirtipes
P. fontanum
Standard
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

Text-fig. 8. Inversions on standard IS.

Cytological Synopsis

The % TCL of the seven forms (Table II) reveals no great differences that would call duplication-deficiencies, translocations, or pericentric inversions for explanation. Interspecific inversions shifting the nucleolar organizer or Ring of Balbiani from their standard location have not occurred in any form in this group. Most of the changes were paracentric inversions which in some cases were complicated by heterochromatization. A brief account, with a diagrammatic sketch of each chromosome arm is given below in order to put together the various rearrangements which have occurred on individual standard arm in the evolution of this group of black flies.

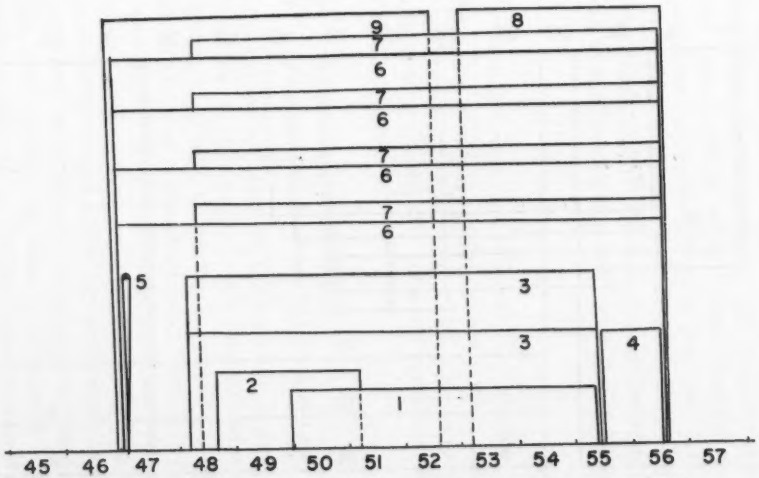
The standard type of IS is maintained in *P. inflatum* (Text-fig. 8) from Scotland and in its relatives in the High Pyrenees (*P. inflatum* aff. 1) and Austria (*P. inflatum* aff. 3). The Alpine form, *P. inflatum* aff. 2, differs from standard in a fixed inversion, *IS-18*, which also occurs as a floating inversion in the High Pyrenees and Austrian forms. The Austrian form *P. inflatum* aff. 3 has a floating inversion IS-19. IS of *P. vigintiquaterni* is modified into *X* and *Y*, the former differing from standard in IS-14 and IS-15 and the latter in IS-16. IS-14.17 is another floating inversion in *P. vigintiquaterni*. IS of *P. fontanum* is differentiated into *X* and *Y*, both sharing the interspecific inversions *IS-1*. In the *Y* chromosome of *P. fontanum* there are three independent inversions, IS-2, IS-3, and IS-4, and a complex inversion, IS-5.6.7. IS of *P. hirtipes* carries a fixed inversion *IS-8.9* and four floating inversions IS-10, IS-11, IS-12, and IS-13.

IL has remained fairly conservative in forms of this group. The only interspecific arrangements on IL are *IL-1* of *P. fontanum* and *IL-8* of *P. inflatum* aff. 2. *P. hirtipes* of Scotland has three floating inversions IL-2, IL-3.4, and IL-5. (Text-fig. 9). IL-7 is a floating inversion seen in *P. inflatum* aff. 1.

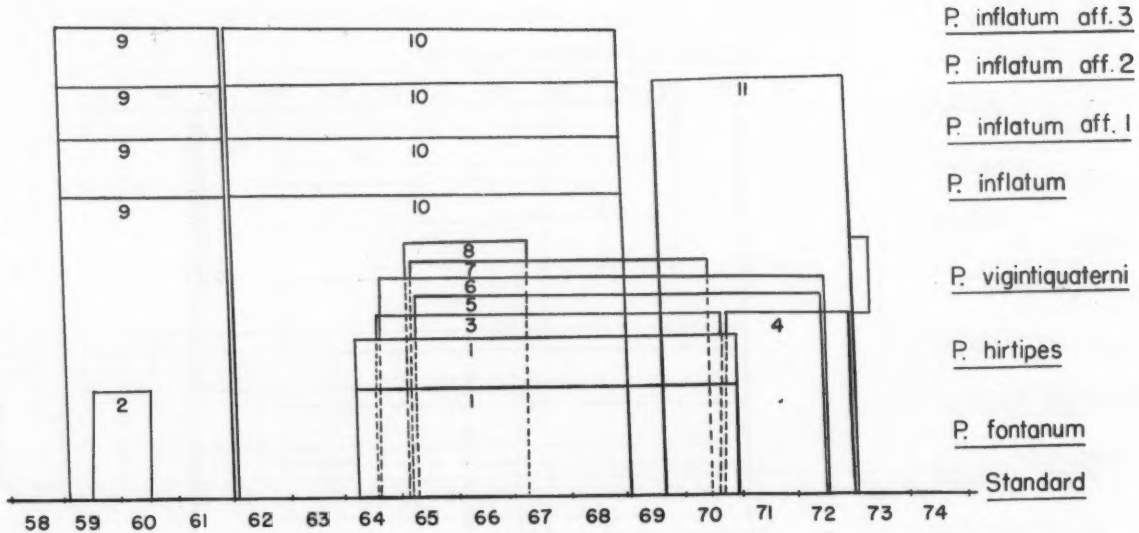
Standard IIS is present in none of the seven forms. *P. fontanum* (Text-fig. 10) differs from standard in an interspecific inversion *IIS-1* and carries a floating inversion IIS-2. *P. hirtipes* and *P. vigintiquaterni* differ from standard in *IIS-3*. *P. hirtipes*, in addition, carries an interspecific inversion *IIS-4*, while *P. vigintiquaterni* shows a heterochromatin-associated non-conformity, IIS-5. IIS of *P. inflatum* from Scotland and its allies from southern Europe bear an interspecific rearrangement, *IIS-6.7*, while *P. inflatum* aff. 3 has another interspecific inversion *IIS-8*. IIS-9 is a floating inversion in *P. inflatum* aff. 3.

Standard IIL is present only in *P. fontanum* (Text-fig. 11). *P. hirtipes* and *P. vigintiquaterni* differ from standard in an inversion step *IIL-1*. IIL-2 is a small floating inversion in *P. hirtipes* from Scotland. IIL-3 is a common floating inversion in *P. vigintiquaterni* which also carries two rare floating inversions, IIL-4 and IIL-5.6.7.8, on the two constituents of a complex heterozygous inversion. *P. inflatum* and related forms have a fixed inversion *IIL-9*. IIL-10 is a floating inversion in *P. inflatum* larvae from Scotland and *P. inflatum* aff. 1 from the High Pyrenees, but is fixed in the Alpine and Austrian forms. *IIL-11* is a fixed inversion in the Alpine form, *P. inflatum* aff. 2.

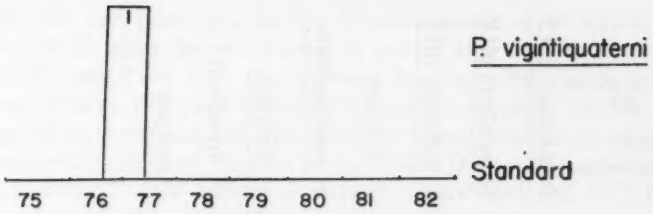
Text-fig. 9. Inversions on standard IL.



Text-fig. 10. Inversions on standard IIS.



Text-fig. 11. Inversions on standard IIL.



Text-fig. 12. Inversions on standard IIIS.

The only inversion on IIIS is IIIS-1 seen heterozygously in *P. vigintiquaterni* (Text-fig. 12).

All the European species in this group of black flies are standard for IIIL (Text-fig. 13). The only interspecific inversion on this arm is *IIIL-1* seen in the North American species, *P. fontanum*. *P. hirtipes* has two floating inversions IIIL-2 and IIIL-3 and an extra heterochromatic piece (IIIL-4). *P. vigintiquaterni* has three floating inversions on this arm, IIIL-5.6, IIIL-7.8.9.10, and IIIL-11. IIIL of *P. inflatum* from Scotland carries a floating inversion, IIIL-12, and a reverse repeat IIIL-13 (seen also in one larva from the Grenoble region). IIIL-14.15 is a floating inversion seen in *P. inflatum* aff. 3 (Text-fig. 13.

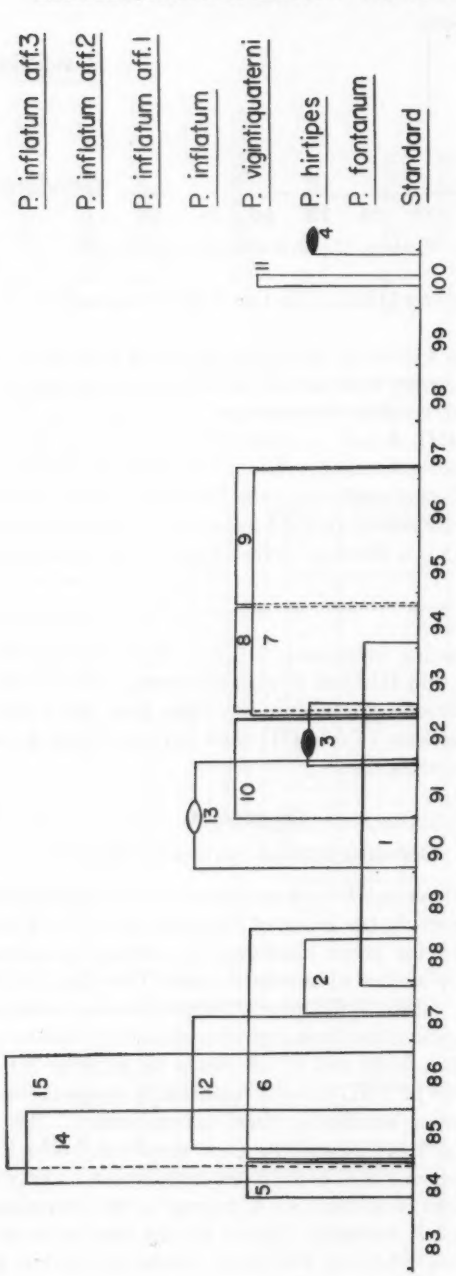
Statistics

The common floating inversions, IIS-2 of *P. fontanum*, IS-10, IS-11, and IL-2 of *P. hirtipes*, and IIL-3 of *P. vigintiquaterni*, were tested for conformity with the Hardy-Weinberg distribution. The gene sequence frequencies of these common inversions (Table III) show no significant departure from the Hardy-Weinberg expectation.

Discussion

Distribution of Inversion Breaks

The location of inversion breaks on chromosomes is generally considered to be random. However, in the group of black flies with which we are concerned, there is a tendency for break clustering in certain chromosome segments. The diagrammatic sketches of standard arms (Text-figs. 8–13) show that all chromosome arms, except IIIS, have undergone many inversion changes and that certain break points on these arms are shared by two or more inversions. For example, sections 3*c*–3*d* and 17/18–18*p* of IS, sections 55*c* and 56*c* of IIS, 65*p* of IIL, and 84*d* of IIIL show a remarkable preponderance of inversion breaks, over the other sections in these chromosomes. When the inversion breaks on a given arm are plotted for each species individually, the tendency for break clustering becomes even more conspicuous. To cite the instance of IS, all four breaks in section 3*c*–3*d* belong to the inversions (*IS-9*, IS-10, IS-12, and IS-13) of *P. hirtipes*. Out of the six inversions on the *Y* chromosome of *P. fontanum*, IS-5 and IS-7 have breaks at section 10/11, IS-5 and IS-6 have breaks at 11*c*, and IS-3 and IS-4 have breaks at section 17/18.



Text-fig. 13. Inversions on standard IIIL.

Similarly, the two inversions on the *X* chromosome of *P. vigintiquaterni*, IS-14 and IS-15, share the break point in section 18*p*. A glance at the text figures 9–13 will prove that the inversion breaks pertaining to individual species on all arms, except IIIS, follow the pattern described for IS.

Clustering of inversions and coincidence of break points, such as observed in our material, have been reported in *Drosophila* (8, 21), *Chironomus* (1, 25), and black flies (10), and various explanations have been put forth for such situations (17, 21, 32). Novitski (21) attributes break clustering on chromosomes to the induction of breaks initiated by pairing tension in the unpaired regions in chromosomes which are heterozygous for an existing inversion. Rothfels and Fairlie (25) have analyzed the causes underlying the phenomenon of non-random breakage of chromosomes and have found themselves in agreement with the Novitski hypothesis. The pattern of break distribution in our material also seems to suggest that the positions of new inversion breaks on chromosomes, at least in some cases, are influenced by the existing inversions.

Sex-determining Mechanism

All exhaustively studied forms discussed in this paper possess a distinct cytological sex-determining mechanism. The heterozygosity in the male sex, resulting from an *XX–XY* sex-determining mechanism, is familiar from early *Drosophila* studies and has been reported in *Chironomus* (5) and several species of black flies (24). In certain black flies the sex-associated heterozygosity is expressed by a failure of pairing in the centromere region of one of the chromosomes as in *Cnephia mutata* (3) or by an extra heterochromatic "inclusion" as in *Eusimulium aureum*, form D (10), while a great many *Prosimulium* species possess simple or complex "sex loops" (24).

In *P. hirtipes* there are forms having no cytological sex-determining mechanism (forms from Scotland) and there are others which do have a cytological sex-determining mechanism (forms from England), which, however, does not involve cytological heterozygosity in males. The fact that differentiated sex chromosomes occur in these latter populations at all was lost to us initially since the only common heterozygous inversion in this form, IS-10, was found in both males and females. But in the course of our analysis of this inversion, we found that the homozygous sequence for IS-10 (Fig. 8) was restricted to the female sex (Table V). The total absence of IS-10/IS-10 males could be due to one of the two reasons: the sequence IS-10 carries a recessive gene which when homozygous, is lethal in males. This hypothesis presupposes that the lethal effect of the homozygous inversion is counteracted by some other factor in the females and that the inversion IS-10 is not directly related to sex determination. The alternative explanation not only maintains the presence of distinct sex chromosomes, but also necessitates the occurrence of two types of *X* chromosomes, i.e. IS (X_0) and IS-10 (X_1), and of a genetic *Y* which is cytologically undifferentiated (Y_0). To substantiate the latter assumption there should be females with three different

genotypes in the population: IS/IS (in which the X is undifferentiated, X_0X_0), IS/IS-10 (in which one X chromosome is differentiated, X_0X_1), and IS-10/IS-10 (in which both X chromosomes are differentiated, X_1X_1). Similarly males with two genotypes could be expected: IS/IS (where X and Y are cytologically undifferentiated, X_0Y_0) and IS/IS-10 (where X is differentiated from Y, X_1Y_0). Table V shows that this is the case in *P. hirtipes* populations from England.

The sex-bound inversion of *P. inflatum* from Scotland confronted us with difficulty in unravelling it. Although the non-pairing of the two constituents at the centromere region of chromosome I in all males suggests that this region is involved in the sex-determining mechanism, the nature of the heterozygosity associated with male sex is by no means established satisfactorily. The presence of a "normal" centromere region in all females, however, indicates that the rearrangement associated with male sex is not on the X but on the Y chromosome. The only segment, which is well identified as being inverted, in the Y chromosome extends from $21'c$ to $23p$ (Fig. 25). Some nuclei show reverse pairing of the four heavy bands in section 20′; but their inconsistent pairing behavior offers difficulty in deciding the length of the segment as well as the type of heterozygosity involved in the sex-determining mechanism. A normal nucleolus, represented in cytological preparations by an irregular gap, is seen only on the X chromosome while the probable "nucleolar organizer" on Y is always represented by a heavy heterochromatic band. The information on forms related to *P. inflatum* from Continental Europe is incomplete since the larvae were all too immature to give us information on their gonadal sex. However, of the many samples of larvae we examined, none of the Alpine, the High Pyrenees, or the Austrian forms showed the sex-restricted heterozygosity at the centromere region, characteristic of *P. inflatum* males from Scotland.

The cytological sex-determining mechanism in *P. vigintiquaterni* involves basically three independent inversions on IS. The X chromosome differs from standard IS in two rearrangements, IS-14 and IS-15, and the Y carries an inversion, IS-16, involving just two bands. All male larvae from Czechoslovakia, and probably those from Central France, carry these three inversions. Inversion IS-14.17, seen in a single female from Czechoslovakia, obviously represents another step in the differentiation of the X chromosome. The inversions limited to X and that in Y in *P. vigintiquaterni* seem to be the various steps towards ensuring greater structural differences between X and Y.

In *P. fontanum* nearly two-thirds of the length of IS and the entire expanded region of chromosome I are involved in the sex-determining mechanism. Both X and Y in *P. fontanum* differ from standard basically by a simple inversion, *IS-1*. The Y chromosome has subsequently acquired six more rearrangements, one of which, somewhat arbitrarily described as inversion IS-2, being akin to the unresolved sex rearrangement encountered in *P. inflatum* males from Scotland. The case of *P. fontanum* is an extreme one in which the sex chromosomes are greatly differentiated by various inversions which, by preventing crossing over, would preserve the genetically isolated

gene blocks in both *X* and *Y*. Unlike some species of black flies in which two or more *Y* chromosomes, representing the various stages in the process of differentiation, are detected (24), *P. fontanum* has only one type of *Y* chromosome which obviously is the end product of a long evolutionary history.

The sex-associated chromosome dimorphism in all adequately studied species in this group is consistently in chromosome I. The heterozygosity in males in *P. inflatum* takes in the entire centromere region of chromosome I and extends well beyond the nucleolar organizer on IL. The rearrangements involving the modification of chromosome constituents into *X* and *Y* are confined to IS in *P. hirtipes* and *P. vigintiquaterni* while in *P. fontanum*, in addition to IS, the entire expanded region of chromosome I is also involved in the cytological sex-determining mechanism.

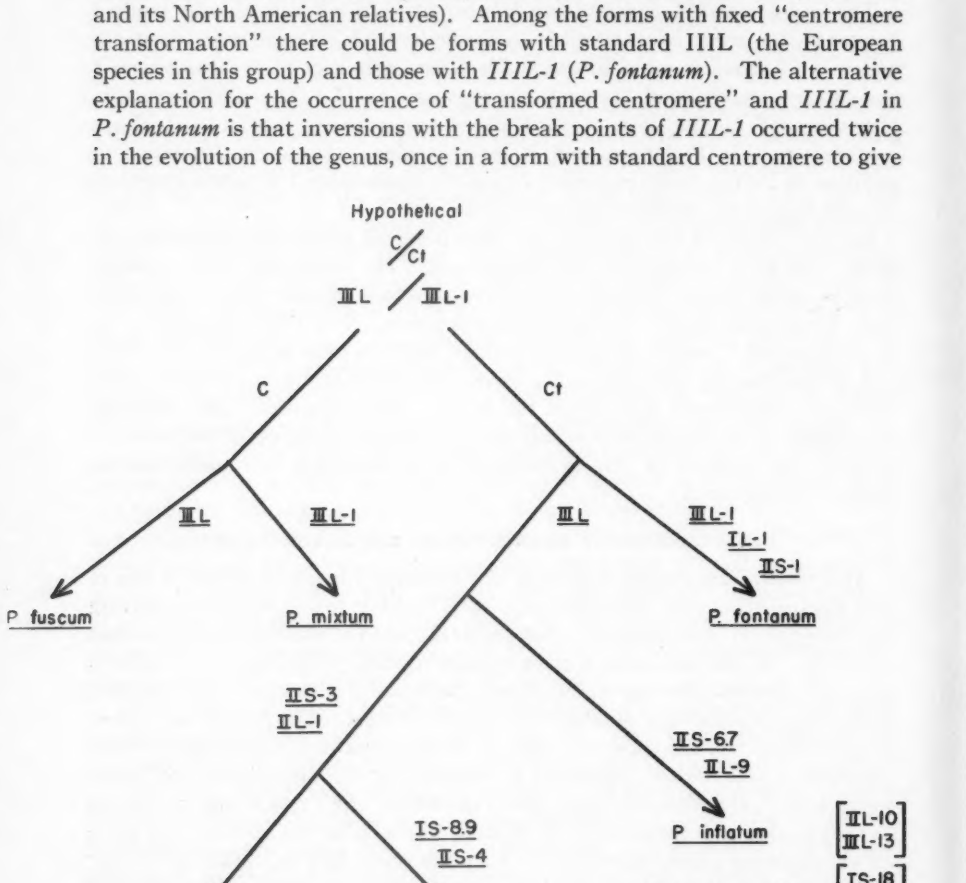
Larval samples of the same species from different geographic areas differ in their cytological sex-determining mechanism. The population of *P. hirtipes* from Scotland has no cytologically differentiated sex chromosomes while those from England have two types of *X* chromosomes. The High Pyrenees form, *P. inflatum* aff. 1, which is identical with *P. inflatum* from Scotland in its fixed and several floating rearrangements, shows no heterozygosity in the centromere region of chromosome I in any of the larvae. The failure to detect the nonconformity in the centromere region in any of the 44 larvae examined seems to indicate the absence of this cytological sex-determining mechanism in the High Pyrenees form.

Phylogenetic Relationships of the Seven Forms

All *Prosimulium* species studied in this laboratory could be placed in one of two groups depending upon the type of IIIL they carry: one group having basically the IIIL sequence of *P. fuscum* (IIIL) and the other that of *P. mixtum* (*IIIL-1*). All the European species included in this report and over 20 North American *Prosimulium* species analyzed cytologically in this laboratory possess IIIL which is essentially similar to that of *P. fuscum*. The *P. mixtum* type of IIIL (*IIIL-1*) has been observed only in *P. fontanum* and in a few other North American species closely related to *P. mixtum*, e.g. *P. saltus* Stone and Jamnback, and *P. rhizophorum* Stone and Jamnback. As regards the remaining five arms, the gene sequence of *P. mixtum* seems to be the most central since all chromosome arms, except IIIL of *Prosimulium* species, could be directly derived from those of *P. mixtum*. One may, therefore, postulate a hypothetical ancestral *Prosimulium* which combines the IIIL of *P. fuscum* with the remaining arms of *P. mixtum*. Species with such cytological characteristics are not observed in *Prosimulium*. A triploid species, *Prosimulium ursinum* Edwards (*Prosimulium* "O" of Rothfels (24)), however, does have the basic gene sequence of the five arms of *P. mixtum* and the IIIL of *P. fuscum*. These observations support the choice of the hypothetical ancestor as a form with the *P. fuscum* type of IIIL and with the other arms of *P. mixtum*.

The various chromosome combinations observed in the North American and European *Prosimulium* species can be reconciled most readily if the ancestral form were heterozygous for "centromere transformation" and inversion *IIIL-1*,

(Text-fig. 14). From this ancestor, forms with standard centromere as well as those with "transformed centromere" could be obtained. In the group with standard centromere, there could be forms with fixed *IIIL-1* (such as *P. mixtum* and related North American species) and forms with standard IIIL (*P. fuscum* and its North American relatives). Among the forms with fixed "centromere transformation" there could be forms with standard IIIL (the European species in this group) and those with *IIIL-1* (*P. fontanum*). The alternative explanation for the occurrence of "transformed centromere" and *IIIL-1* in *P. fontanum* is that inversions with the break points of *IIIL-1* occurred twice in the evolution of the genus, once in a form with standard centromere to give



Text-fig. 14. Chart showing the phylogenetic interrelationship of the seven forms of *Prosimulium* with "transformed centromere" region. All fixed inversions (along the arrows) and the significant floating inversions (in brackets) of each form are shown in the chart. C, standard centromere; Ct, transformed centromere.

rise to *P. mixtum* and its North American relatives, and again, as a "mimic" inversion in a form with "transformed" centromere to give *P. fontanum*.

In the evolutionary line of the European species, one would expect, nearest to the ancestor, a species in which all arms are standard (hypothetical) and the centromere transformation is fixed. None of the North American or European species we have examined fits that description. *P. fontanum*, however, is close to the hypothetical ancestor from which it differs only by two inversions *IL-1* and *IIS-1*. Granted the existence (past or present) of such an intermediate form, the chain of events from this point would branch in two directions: on one side, inversions *IIS-3* and *IIL-1* would give rise to a group of *Prosimulium* of which the species we have on hand are *P. vigintiquaterni* and *P. hirtipes*. The former has undergone three inversions on IS (IS-14, IS-15, and IS-16) in order to modify its constituents into *X* and *Y*. The latter, *P. hirtipes*, bifurcates from this evolutionary line by acquiring a two-step complex inversion *IS-8.9* and an additional inversion, *IIS-4*.

On the other branch, from the intermediate form, *P. inflatum* of Scotland and its relatives in Southern Europe could be derived by the fixation of *IIS-6.7* and *IIL-9*, common to all these forms. *P. inflatum* aff. 1, while identical with *P. inflatum* in its predominant gene sequences and in the floating inversion IIL-10, in addition, has the floating inversion IS-18, seen also in *P. inflatum* aff. 3. *P. inflatum* aff. 2 of the Alps and Central France can be derived from *P. inflatum* aff. 1 by the fixation of *IS-18* and *IIL-10* and by the addition of two or more inversions *IL-8* and *IIL-11*. *P. inflatum* aff. 3 could be derived from *P. inflatum* aff. 1 by the fixation of *IIL-10* and by the addition of one more rearrangement, *IIS-8*.

The Taxonomic Status of the Seven Forms with a Transformed Centromere

There is no doubt that *P. fontanum*, *P. hirtipes*, *P. vigintiquaterni*, and *P. inflatum* from Scotland are distinct species as they differ in the ordinary taxonomic criteria (7, 20, 30), as well as in their cytological features. All these species differ from one another in many fixed chromosome rearrangements and in the details of their specific chromosome polymorphism. Furthermore, in those forms which occur together, i.e. *P. hirtipes* and *P. inflatum* from Scotland, the cytological evidence is quite clear that interbreeding does not occur.

One problem which arises is the relation between *P. hirtipes arvernense* Grenier and *P. vigintiquaterni* Enderlein. Forms submitted under these two names from France (*P. hirtipes arvernense*) and Czechoslovakia (*P. vigintiquaterni*) are cytologically identical; and since the Czechoslovakian material, according to Novak (personal communication), conforms to Enderlein's description (13), it would seem that the name *P. vigintiquaterni* has priority and that *P. hirtipes arvernense* should be dropped. In any case it is clear from the cytological evidence that this form should be given species rank rather than the subspecific status as proposed by Grenier (15, 16).

Other difficulties arise in regard to the relation between *P. inflatum* from Scotland and the forms described as *P. inflatum* aff. 1, *P. inflatum* aff. 2, and *P. inflatum* aff. 3 from the mountains of Southern Europe. Taking this group as a whole, all these forms have in common the interspecific inversions *IIS-6.7* and *IIL-9*. *P. inflatum* aff. 1 of the High Pyrenees, in addition, shares a floating inversion IIL-10 with *P. inflatum* of Scotland. Other floating inversions are restricted to *P. inflatum* or *P. inflatum* aff. 1 of which IS-18 of *P. inflatum* aff. 1 is significant as it is present also in the Alpine and Austrian forms. The absence of any interspecific inversion difference and the sharing of at least one floating inversion indicate very strongly that *P. inflatum* and *P. inflatum* aff. 1 are, in fact, one and the same species.

In *P. inflatum* aff. 2 the floating inversions *IIL-10* and *IS-18* have become fixed and it differs from *P. inflatum* aff. 1 by the acquisition of two more fixed inversions *IL-8* and *IIL-11*. It might be speculated that the heterozygotes for *IL-8* and *IIL-11* exist among forms occupying intermediate areas between the Alps and the High Pyrenees and that the two forms are members of an interbreeding population in which geographic substitution of inversions causes the heterozygotes to shade into homozygosity. This possibility is counterindicated to some extent by the observation that both *P. inflatum* aff. 1 and *P. inflatum* aff. 2 larvae occurred together in one collection from the Grenoble region. Although more extensive study of collections from yet other localities is desirable, the provisional conclusion must be that the form referred to as *P. inflatum* aff. 2 is a species reproductively and biologically distinct from *P. inflatum*, inclusive of *P. inflatum* aff. 1.

P. inflatum aff. 3 from Austria also exhibits cytological features common to this group. The only rearrangement in which the Austrian form differs from *P. inflatum* aff. 1 is *IIS-8*, which is not seen heterozygously or homozygously in any other form. On the other hand, *P. inflatum* aff. 3 shares with *P. inflatum* aff. 1 the floating inversion IS-18. Since our information on this form comes from a limited supply of specimens from only one locality and is not supplemented by morphological study, it would be precipitate to assign species status to *P. inflatum* aff. 3 solely on the absence of *IIS-8* heterozygotes. The present conservative conclusion must, therefore, be that *P. inflatum* aff. 3 is a geographic variant of *P. inflatum* aff. 1 in which inversion IS-18 has remained floating; but this tentative conclusion must be confirmed by analysis of other populations and by co-ordinated taxonomic study.

Summing up our cytological information on these forms, *P. inflatum* from Scotland, *P. inflatum* aff. 1 from the High Pyrenees, and, probably, *P. inflatum* aff. 3 from Austria are different geographic populations of the same species, while *P. inflatum* aff. 2 of the Alps forms a distinct species on the merit of its cytological integrity.

The taxonomic study (7) of *Prosimulium* from the High Pyrenees, Austria, and the Alps has shown that these forms, variously called *P. hirtipes* (Fries), *P. rufipes* (Meigen), and *P. galli* Edwards, are distinct from the North European species, *P. hirtipes* (Fries), and that they form a rather heterogeneous group related, in their morphological features, to *P. inflatum*. Very recently

L. Davies (personal communication) has examined the residues of *P. inflatum* aff. 1 and *P. inflatum* aff. 2 larvae from which our cytological preparations were made, and has reported that *P. inflatum* aff. 1 is indistinguishable from *P. inflatum*, and that *P. inflatum* aff. 2 is the distinctive species *P. rufipes*, thereby supporting our cytological conclusions on these forms.

Summary

A group of seven forms of *Prosimulium* having a "transformed" centromere region in chromosome I was analyzed cytologically. One member of this group was collected from Ontario and the remaining six were received from various parts of Europe.

Five chromosome arms of *P. mixtum* and the IIIL arm of *P. fuscum* were chosen as standard with which the respective arms of each form were compared.

Photographic plates, showing the six standard arms and the altered segments of chromosomes in each form, have been presented.

The gross morphological features and the fixed and floating band differences in chromosomes have been shown in the idiogram constructed for each form.

A phylogenetic chart, based on the fixed rearrangements in the band sequence of these forms, was drawn up and the taxomonic status of the seven forms has been discussed.

Acknowledgments

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References

1. ACTON, A. B. Larval groups in the subgenus *Chironomus* Meigen. Arch. Hydrobiol. **50**, 64–75 (1955).
2. BASRUR, V. R. Inversion polymorphism in the midge *Glyptotendipes barbipes* (Staeger). Chromosoma, **8**, 597–608 (1957).
3. BASRUR, V. R. Cytotaxonomic studies in black flies and midges. Ph.D. Thesis, University of Toronto, Toronto, Ontario. 1957.
4. BAUER, H. and BEERMANN, W. Die Polytänie der Riesenchromosomen. Chromosoma, **4**, 630–648 (1952).
5. BEERMANN, W. Geschlechtsbestimmung and Evolution der genetischen Y—Chromosomen bei *Chironomus*. Biol. Zentr. **74**, 525–544 (1955.)
6. CONGER, A. D. and FAIRCHILD, L. M. A quick-freeze method for making smear slides permanent. Stain Technol. **28**, 281–283 (1953).
7. DAVIES, L. A new *Prosimulium* species from Britain and a re-examination of *P. hirtipes* Fries from the holarctic region. (Diptera, Simuliidae). Proc. Roy. Entomol. Soc. London, **26**, (1–2), 1–10 (1957).
8. DOBZHANSKY, T. and EPLING, C. Contributions to the genetics, taxonomy and ecology of *Drosophila pseudoobscura* and its relatives. Carnegie Inst. Wash. Publ. No. 554, 1–183 (1944).
9. DUNBAR, R. W. The salivary gland chromosomes of two sibling species of black flies included in *Eusimulium aureum* Fries. Can. J. Zool. **36**, 23–44 (1958).
10. DUNBAR, R. W. The salivary gland chromosomes of seven forms of black flies included in *Eusimulium aureum* Fries. Can. J. Zool. **37**, 495–525 (1959).

11. EDWARDS, F. W. On the British species of *Simulium*. I. The adults. Bull. Entomol. Research, **6**, 23–42 (1915).
12. EDWARDS, F. W. New species of Palaearctic Simuliidae in the British Museum (Diptera, Nematocera). Ann. Mag. Nat. Hist. **7**,(9), 141–143 (1921).
13. ENDERLEIN, G. Beitrag zur Kenntniss der Prosimuliiden and Hellichiinen. Sitzber. Ges. naturforsch. Freunde Berlin, 224–226 (1929).
14. FRIES, B. F. Monographia Simuliarum sveciae. Observationes entomoligacae, **1**, 5–20 (1824).
15. GRENIER, P. Notes morphologiques et biologiques sur quelques Simulies nouvelles pour la faune française. Bull. soc. entomol. France, **52**, 66–69 (1947).
16. GRENIER, P. Contribution à l'étude biologique des Simuliides de France. Physiol. Comparata et Oecol. **1**, 165–330 (1949).
17. IVES, P. T. The importance of mutation rate genes in evolution. Evolution, **4**, 236–252 (1950).
18. KUNZE, E. Untersuchungen uber die Paarungsaffinitat bei Riesenchromosomen. Chromosoma, **5**, 501–510 (1952).
19. KUNZE, E. Artunterschiede im Bau der Riesenchromosomen in der Gattung *Simulium* Latr. Österr. Zool. Z. **4**, 23–32 (1953).
20. NOVAK, V. Note on the black-flies of Czechoslovakia Vestnik Ceskoslo-venske Zool. Spolecnoste Acta Soc. Zool. Bohemoslov. **20**, 224–248 (1956).
21. NOVITSKI, E. Chromosome variation in *Drosophila athabasca*. Genetics, **31**, 508–524 (1946).
22. PURI, I. M. On the life history and structure of the early stages of the Simuliidae (Diptera Nematocera). Parasitology, **17**, 335–369 (1925).
23. ROTHFELS, K. H. and DUNBAR, R. W. The salivary gland chromosomes of the black fly *Simulium vittatum* Zett. Can. J. Zool. **31**, 226–241 (1953).
24. ROTHFELS, K. H. Black flies: siblings, sex and species grouping. J. Heredity, **47**, 113–122 (1956).
25. ROTHFELS, K. H. and FAIRLIE, T. W. The non-random distribution of inversion breaks in the midge *Tendipes decorus*. Can. J. Zool. **35**, 221–263 (1957).
26. ROTHFELS, K. H. Unpublished data.
27. SMART, J. The classification of Simuliidae. Trans. Roy. Entomol. Soc. London, **95**, 463–532 (1945).
28. SOMMERMAN, K. M., SAILER, R. I., and ESSELBAUGH, C. O. Biology of Alaskan black flies (Simuliidae, Diptera). Ecol. Monographs, **25**, 365–385 (1955).
29. STONE, A. and JAMNBACK, H. A. The black flies of New York State. (Diptera, Simuliidae). Bull. N.Y. State Musuem, **349**, 1–144 (1955).
30. SYME, P. D. and DAVIES, D. M. Three new Ontario black flies of the genus *Prosimulium* (Diptera, Simuliidae). Part 1; description, morphological comparison with related species and distribution. Can. Entomologist, **90**, 697–719 (1958).
31. TWINN, C. R. The black flies of eastern Canada (Simuliidae, Diptera). Can. J. Research (D), **14**, 97–150 (1936).
32. WHITE, M. J. D. Animal cytology and evolution. Cambridge University Press, London, 1954.
33. ZIMRING, R. A comparative study of the salivary gland chromosomes of five related black fly species. M.A. Thesis, University of Toronto, Toronto, Ontario. 1953.

TRIPLOIDY IN NATURAL POPULATIONS OF THE BLACK FLY CNEPHIA MUTATA (MALLOCH)[1]

V. R. Basrur and K. H. Rothfels

Abstract

Populations of *Cnephia mutata* in southern Ontario contain both diploid and triploid individuals. The diploid form is bisexual and lacks chromosomal polymorphism except for a rearrangement involved in its cytological sex determining mechanism. The triploids are parthenogenetic; they produce female progeny only and are highly heterozygous for inversions. The banding pattern of the salivary gland chromosomes of diploids and triploids is very similar; the identical standard sequence occurs in both. The complete lack of autosomal inversions in diploids contrasted with their abundance in triploids indicates that effective genetic exchange does not occur between the two forms; they are reproductively completely isolated, although opportunity for cross-mating would seem to exist. The origin of polyploid parthenogenetic forms in black flies is discussed and the view is favored that they are autopolyploid and automictic.

Introduction

In the course of a general cytological survey of black fly species begun in this laboratory several years ago, an unusual situation was incurred in a member of the genus *Cnephia* in which both diploid and triploid forms were found. These occurred frequently together in the same stream, and apparently belonged to the same taxonomic species *Cnephia mutata* (Malloch). Preliminary studies indicated that all the triploids were female, whereas the diploids included males and females in approximately equal numbers. This suggested that the triploids are parthenogenetic and produce female progeny only (thelytokous). The intermingling of triploids with diploids in several locations raised interesting problems with regard to the origin of triploids, their mode of parthenogenetic reproduction, and the degree of genetic isolation from the diploids. Accordingly a detailed cytological analysis was begun. The study received further impetus when it was discovered that the case of *Cnephia mutata* was not unique among black flies. Populations of *Prosimulium ursinum* Edw. collected by R. W. Dunbar at Coral Harbour and Pelley Lake, N.W.T., and by Block in Norway, a new species of *Gymnopais* collected at the N.W.T. locations, and samples of *P. macropyga* Rubtzov obtained through G. Carlsson from Norway proved to consist entirely of triploid females. These findings indicate that triploidy has been invented and perpetuated independently several times in black flies and that the detailed analysis of one such case may yield information of general value.

Materials and Methods

Cnephia mutata (Malloch) is very generally distributed in southern and central Ontario, where most of our larvae were collected. Also at hand is cytologically authentic material from northern New York State and from various streams on the east coast of Vancouver Island, B.C. (courtesy of G. E. Shewell).

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The distribution of the species according to Stone and Jamnback (21) includes the "Canadian, Transition and Upper Austral zones from Alaska and Labrador to California, Wyoming, Arkansas and Alabama". The northern limits of the range are in doubt since records prior to 1952 must be suspected of confusion with the closely related *C. emergens* St. (20). Thus, at least some of the Alaskan records are not authentic (footnote by Stone in 15, 16) and all the larval material which we have from Churchill, Manitoba, is morphologically *C. emergens* and cytologically* distinct from *C. mutata*. Shewell (14) apparently has evidence that *C. mutata* as well as *C. emergens* occurs at Churchill and that Hocking and Richards (9) reports from Goose Bay, Labrador, are authentic, for he shows *C. mutata* distributed throughout the northern Transition Region from Churchill east to Newfoundland.

Table I shows the collection dates, sex, and ploidy of *C. mutata* larvae taken over the past 5 years from various locations in central and southern Ontario and from one location in British Columbia. The degree of ploidy was determined in additional larval samples taken from various streams not listed in Table I, particularly from many sites in the environs of Chalk River, Ontario, where only triploids were found (collections from April 20 on only). On the other hand complete cytological analyses were carried out only on populations from the last three streams listed in Table I.

TABLE I

Location, collection dates, ploidy, and sex of *C. mutata* larvae

Location	Date	Total number of larvae	Ploidy	Sexed larvae ♂	Sexed larvae ♀
Credit River, Ont.	6.4.1952	34	3*n*		18
Beaver River, Grey Co., Ontario	20.4.1952	19	3*n*		16
		3	2*n*		1
Silver Creek, Collingwood Twp., Ont.	20.4.1952	20	3*n*		20
Etobicoke Creek, Long Branch, Ont.	23.4.1953	5	3*n*		1
Etobicoke Creek, Victoria, Peel Co., Ont.	30.4.1952	95	3*n*		95
		5	2*n*	1	4
Amable du Fond, Highway 17, Ont.	21.4.1958	6	3*n*		6
Streams, E. Coast, Van. Is., B.C.	July 1955	5	3*n*		
Credit River Trib., Terra Cotta, Ont.	30.3.1955	5	2*n*		
	25.3.1956	22	2*n*	8	8
	12.4.1956	67	2*n*	15	30
	25.5.1956	10	3*n*		10
	13.4.1957	12	2*n*	4	8
Credit River Trib., Inglewood, Ont.	13.4.1955	22	3*n*		7
		3	2*n*		
	12.4.1956	61	3*n*		61
		2	2*n*		2
	15.3.1956	24	3*n*		24
	17.4.1957	53	3*n*		53
		1	2*n*		1
Kahshe River, Washago, Ont.	20.4.1956	10	3*n*		7

*Preliminary studies indicate that *C. emergens* is diploid, that the nucleolus is organized in the base of the *long* arm of the first chromosome (vs. base of *short* arm in *C. mutata*), and that there is a common floating inversion in IIL, while diploid *C. mutata* are monomorphic in all chromosome arms (a rearrangement in the centromere I region of *C. mutata* is associated with sex determination).

Generally the larvae occur on the underside of submerged rocks and trailing foliage in slow-running streams and are associated with *Simulium vittatum* (Zett.), *Prosimulium fuscum* Syme and Davies, and *P. mixtum* Syme and Davies in the spring and with *S. venustum* (Say) later in the season. According to D. M. Davies (6) *C. mutata* is univoltine. This may be true for the diploid population but must be questioned in the case of triploids, since mature larvae could be obtained throughout the spring and summer. The eggs of *C. mutata* diapause till late fall or early winter and young larvae appear in January or early February. In the Terra Cotta stream, larvae collected in February were almost exclusively $2n$ and these attained prepupal condition in late March and early April. The $2n$ forms gradually decreased thereafter and samples taken from mid-April on were composed almost entirely of $3n$ larvae. The same succession pattern was also observed in the Inglewood stream where, however, $2n$ larvae were much scarcer. In certain other localities where $3n$ larvae predominated, occasional $2n$ larvae were still found in late April and early May, clearly indicating an overlap in the life cycles of the two forms.

Though detailed morphological studies were not carried out, certain differences were recognized between the respiratory filaments dissected from histoblasts of mature diploid and triploid larvae (Fig. 1). The 12 respiratory

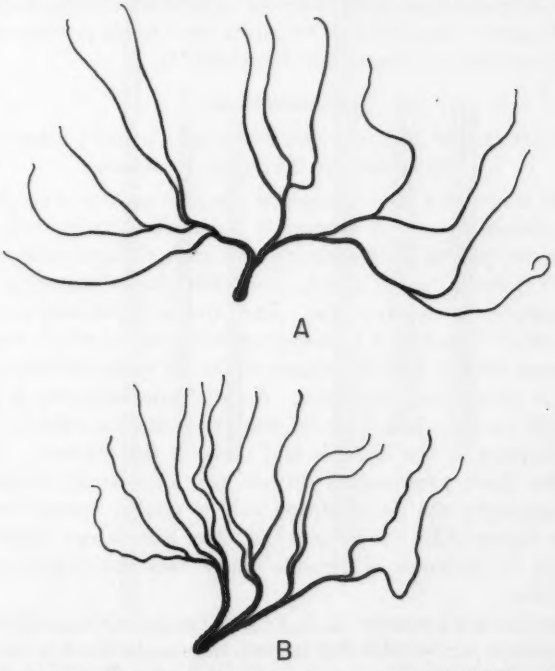

FIG. 1. Respiratory filaments from histoblasts of mature diploid (A) and triploid (B) larvae of *C. mutata*.

filaments in the diploids arise from a long common petiole whereas in the triploids the main trunk branches almost immediately. Further, while the respiratory organs in both carry two main branches with seven and five filaments respectively, the second-order forking of the latter branch is two–three in the triploid but is one–four in the diploid. These differences were consistent in a comparison of 15 diploids and 25 triploids. Stone and Jamnback's (21) figure for *C. mutata* from New York State is like that of our triploids.

The techniques of Rothfels and Dunbar (13) were followed in dissecting, fixing, and staining the salivary gland chromosomes. When immediate processing by the Feulgen technique was not possible, fixed larvae were stored in Carnoy's solution in the refrigerator. Glands on storage developed hard gelatinous cores which interfered greatly with subsequent staining and squashing. This could be overcome by soaking the larvae before hydrolysis for 30 minutes in distilled water when the softened secretion could be removed easily. After hydrolysis and Feulgen staining, a tap water treatment of 15 minutes' duration intensified the stain in the bands and rendered the chromosomes less fragile. In addition to Feulgen slides, orcein mounts were prepared by dissecting the glands directly in *N* HCl and squashing, after staining in aceto-orcein. For study of the nucleolus, larvae were fixed in a mixture of equal volumes of formalin, acetic acid, and ethyl alcohol, in 10 parts of tap water. All slides were made permanent according to the dry-ice method of Conger and Fairchild (3).

Observations

Gross Features of Mitotic and Salivary Gland Chromosomes in Diploid and Triploid *C. mutata*

Contracted stages of gonial mitoses in *Cnephia mutata* show three pairs of metacentric chromosomes. A median or submedian centromere constriction in each of the chromosomes separates the two arms, the arm index of the longest chromosome (I) being approximately one, while that of the other two (II and III) is considerably in excess of one. The mitotic metaphase in the gonad of the $2n$ larvae shows two sets of homologous chromosomes which are somatically paired, whereas similar division stages of the $3n$ forms reveal closely paired homologues in associations of three's. An analogous situation is found in the salivary gland nuclei where each of the chromosomes exhibits two closely paired constituents in the diploids and three in the triploids. Generally in $3n$ larvae the three constituents do not pair completely throughout their length; consequently the $3n$ condition can be readily ascertained in certain chromosome segments by the presence of three non-paired homologues or by the difference in thickness of strands where two homologues pair and the the third is free.

As in other black fly species (1, 7, 12, 13) the individual polytene chromosomes of *C. mutata* can be identified immediately under the low power objective by certain gross features: chromosome I by its over-all length, characteristically enlarged and amorphous centromere region, and nucleolar organizer in the

base of the long arm; chromosome II by the "Ring of Balbiani" (13) in the short arm; and chromosome III by the "frazzle" and "blister" (12) in the short arm. In order to obtain data on the relative sizes of the chromosomes and the position of landmarks (centromeres, nucleolus, Ring of Balbiani) careful measurements of chromosome segments were made in two nuclei from each of five larvae and calculated in per cent total complement length (% TCL) (Table II). The final idiograms were drawn up according to the mean % TCL values, aligning the centromeres in the equator and placing the short arms above and the long arms below (Fig. 2).

TABLE II

Per cent total complement length (% TCL) of diploid and triploid *C. mutata* (Mean of 10 nuclei each)

	Chromosome I				Chromosome II				Chromosome III		
	S–C	C–N	N–L	Total	S–RB	RB–C	C–L	Total	S–C	C–L	Total
Diploid	22.4	7.0	19.5	48.9	7.0	4.0	15.2	26.2	9.8	15.0	24.8
Triploid	25.0	6.4	18.2	49.6	6.4	3.5	14.2	24.1	10.3	15.4	25.7

NOTE: S: "short end; L: "long end"; C: centromere; N: nucleolus; RB: Ring of Balbiani.

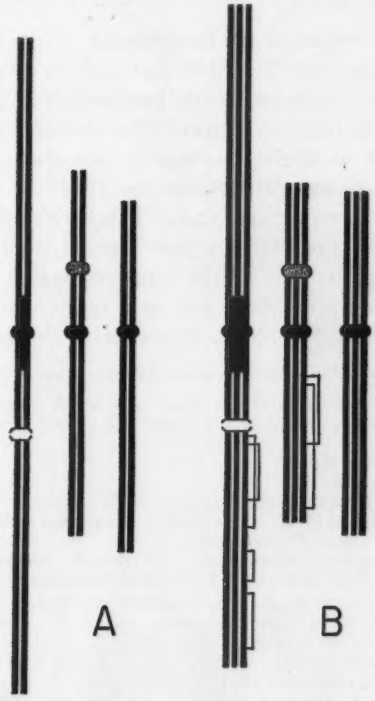

FIG. 2. Salivary gland chromosome idiograms of diploid (A) and triploid (B) *C. mutata*, showing the centromeres in the equator, the nucleolar organizer on IL, and the Ring of Balbiani on IIS. Brackets indicate the approximate positions of floating inversions.

Comparison of the Band Sequence in Diploid and Triploid *C. mutata*

The description of the band sequences is done mainly with the aid of photographic maps. An attempt was made to include at least one well-resolved photograph of each section from the diploid and from the triploid. This proved generally possible for the diploid but difficulties were incurred in the triploid where twisting and complex associations of constituents reduced the number of chromosomes useful for photography. Only one sequence in each chromosome was found in diploid *C. mutata* females and that was chosen as standard. The standard complement was then divided into 100 approximately equal sections numbered 1 to 100 from the "short end" of chromosome I to the "long end" of chromosome III. Since, throughout the complement, the banding pattern of the triploid is very similar to that of the diploid, it was possible to use identical section limits for both. Thus the two forms may be compared readily by reference to major band groups and identically labelled section limits in the photographic maps. The detailed comparison is presented below for each of the three chromosomes.

Chromosome I

Chromosome I, the longest of the complement, is divided into 40 sections, the short arm comprising 1–18 (Figs. 3–9) and the long arm 19–40 (Figs. 7–15). Sections 1–14 of the short arm are clearly homologous in the $2n$ and $3n$ larvae (Figs. 3, 4 vs. 5, 6). The bands in segment 12 are very characteristic in spacing and weight and serve as useful markers in interspecies comparisons with *C. dacotensis* and *C. erimites* (2). Again the bands in segment 14 form a striking pattern of "3 heavy groups" (12). This entire segment is homologous with the basal segment 17 of IS in the generic standard of *Prosimulium* (1).

Sections 15–25, which constitute the expanded region (Figs. 7–9, 15), are highly disorganized and amorphous and as a result could not be compared unequivocally between the two forms. However, on the basis of the occurrence

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Figs. 3–9. Photographic maps of the short arm of chromosome I (IS) from the salivary gland chromosomes of diploid and triploid *C. mutata*. Section limits shown in ink.

Figs. 3, 4. Entire IS of diploid.

Figs. 5, 6. IS of triploid.

Figs. 7. 8, Expanded region of chromosome I in diploid male. The two constituents cross in the region of sections 19–20. The labelled constituent is the *X* chromosome, the unlabelled constituent is the *Y*. C: centromere.

Fig. 9. Expanded region of chromosome I in a triploid. Note pairing of centromeres (C). The upper constituent corresponds to the *X* chromosome of the diploids.

Figs. 10–16. Photographic maps of IL of diploid and triploid *C. mutata*.

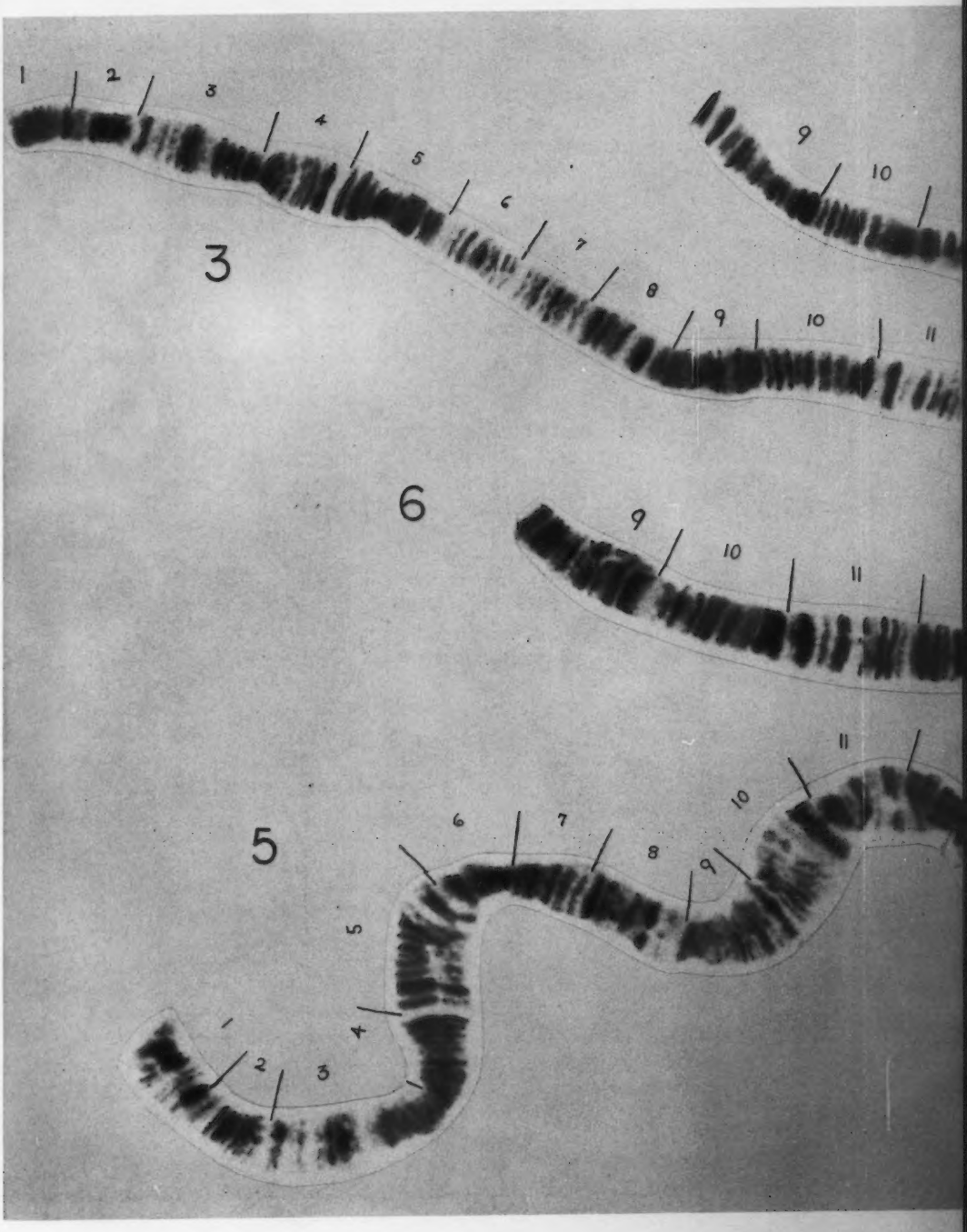
Figs. 10, 11. Entire IL of diploid, showing also by brackets the break points of inversions IL-1 to IL-4 of the triploids.

Figs. 12, 14. IL end of triploid.

Fig. 13. Heterozygous inversion IL-1 in triploid (SSI).

Fig. 15. IL base and expanded region in triploid. C: centromere; N: nucleolar organizer.

Fig. 16. Complex heterozygous inversion IL-3/IL-4 in triploid from Kahshe River. Two constituents are IL-4, the third is IL-3. (*a*) Photograph, (*b*) interpretation of pairing configuration showing three paired segments BC, DE, FG.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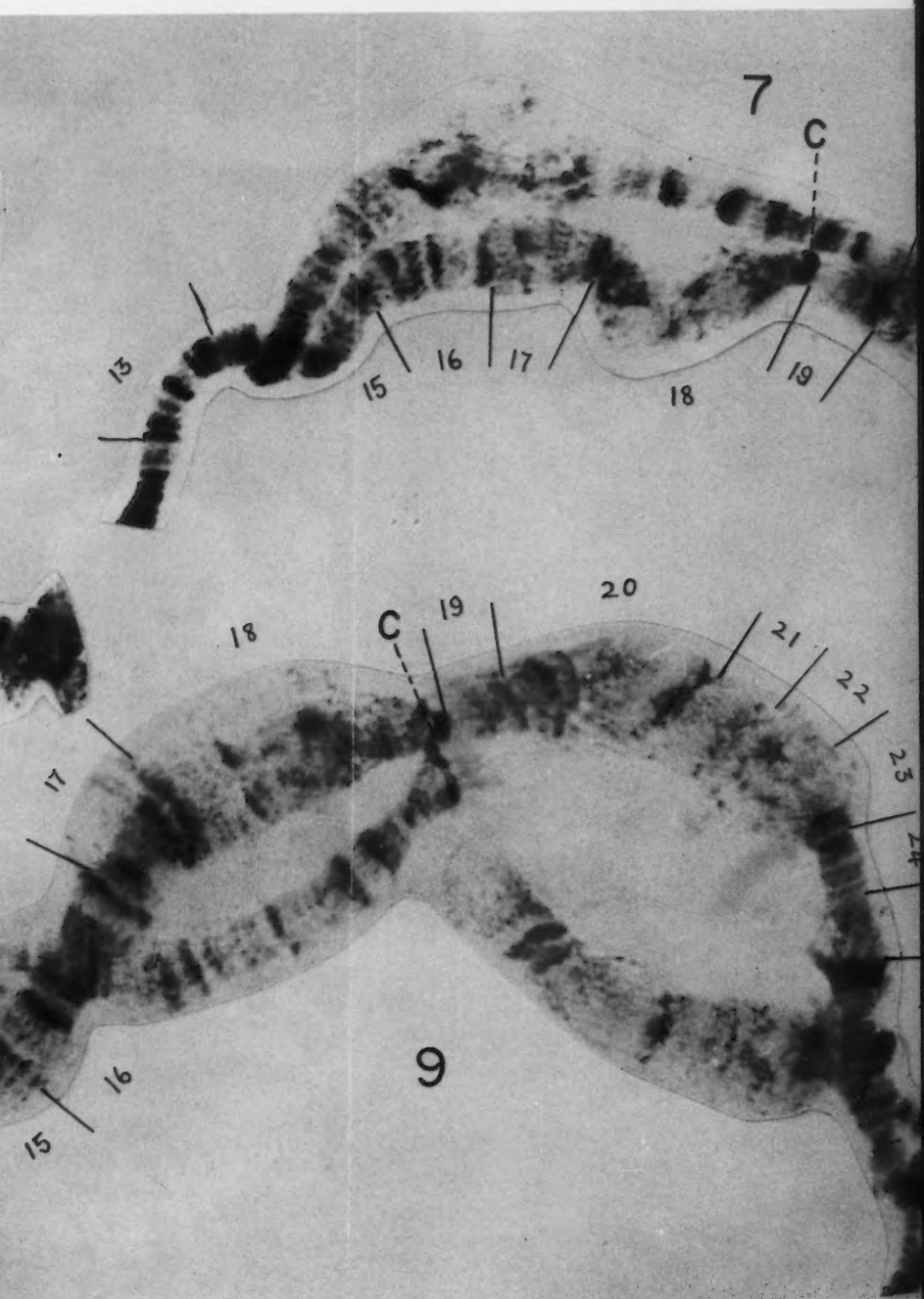
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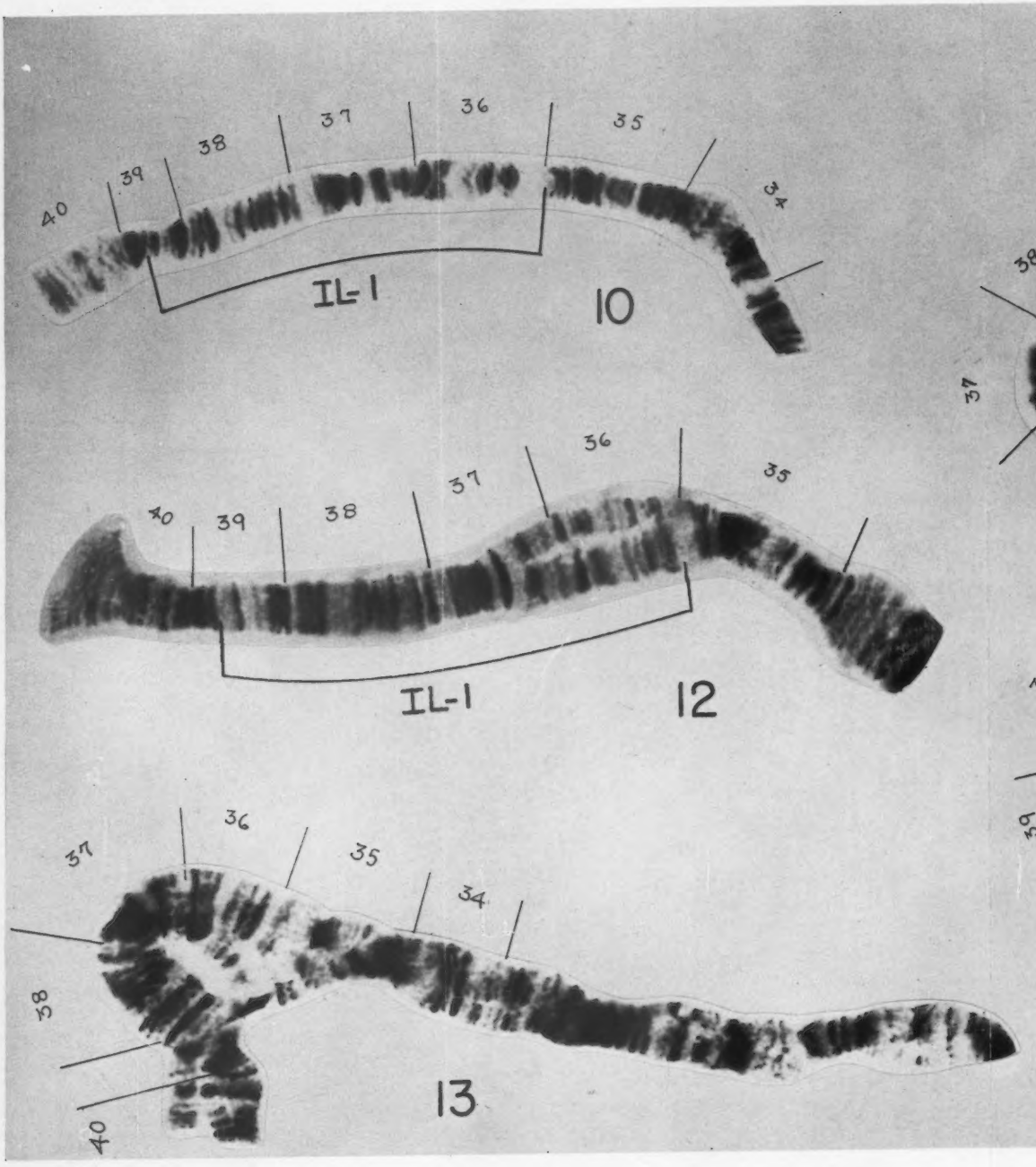
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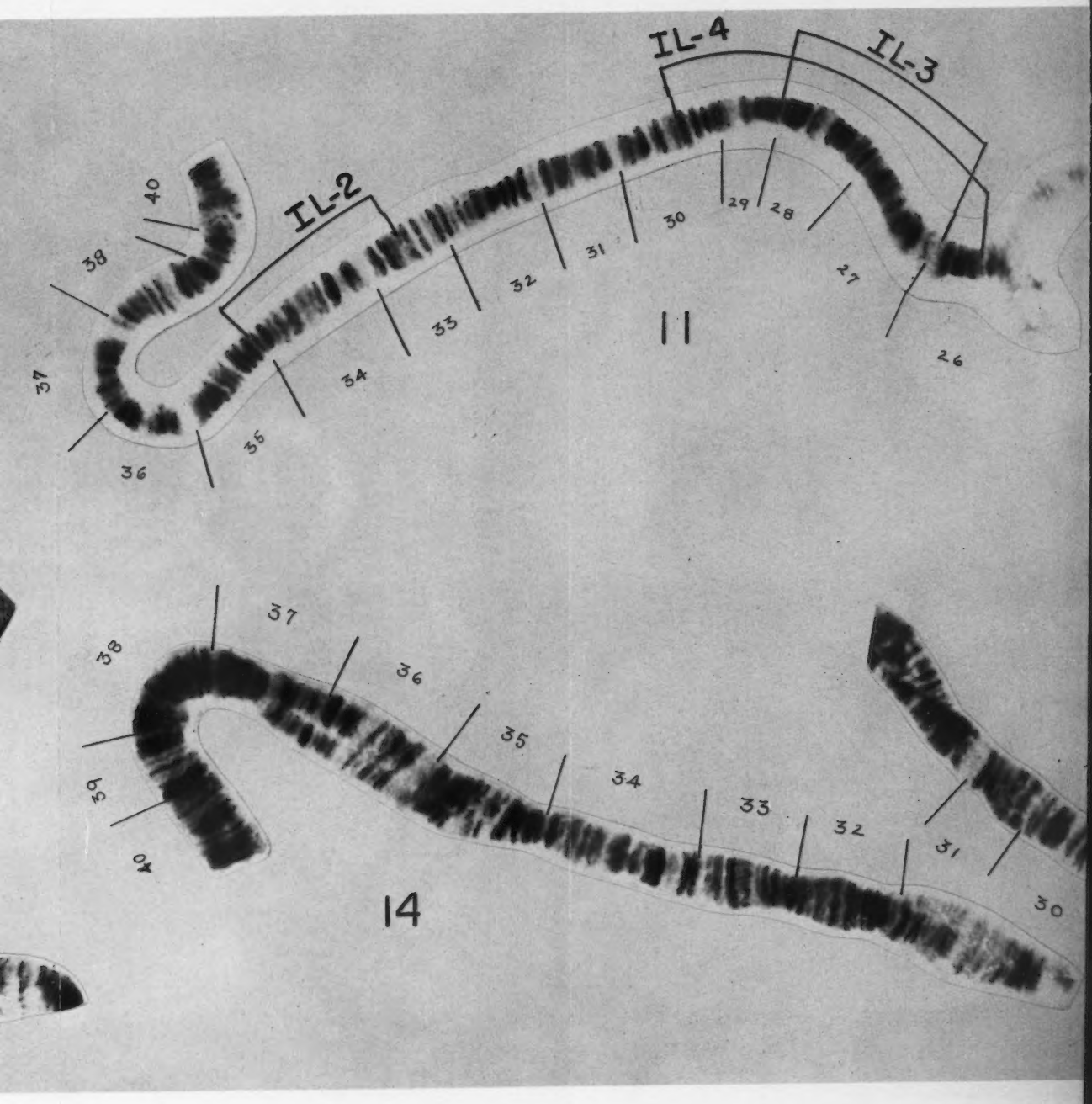
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16a

in both $2n$ and $3n$ individuals of certain very heavily stained disaggregated bands an attempt was made to homologize the expanded regions as indicated by the choice of section limits in Figs. 7, 9, 15. In the expanded region of the $3n$ forms the constituents were always partly or wholly dissociated (Fig. 9). It is not known whether non-pairing is a general property of this region or whether it is due to unresolved structural rearrangements. Within the expanded region both in diploids and triploids the heavily stained terminal band in segment 18 (Figs. 7, 9) is accepted as the centromere because of its tendency to pair in triploids (Fig. 9) and because of its resemblance to well-documented centromeres (ectopic pairing and chromocentric fusion) in other black fly species.

The distal part of section 25 is the site of the nucleolar organizer (Figs. 7–9, 15), and bands in this region tend to disintegrate and flare apart, sometimes resulting in an apparent gap in the chromosome. The first few bands in segment 26 are often heterochromatized as in Figs. 9 and 15. The remaining long arm sections 26–40 are completely homologous in the $2n$ and $3n$ forms as is evident from Figs. 10, 11 vs. 12, 14, and 15. Section 26 ends in a conspicuous band group which serves as a marker in comparison with other *Cnephia* species (2).

Chromosome II

Chromosome II contains 30 sections, 41–71 (Figs. 17–25). The centromere region is taken to be the heavy proximal band in section 52, marked "C" in Figs. 19, 24. It divides the entire chromosome into two clearly unequal parts, the short arm comprising the sections 41–52 proximal, and the long arm ending with section 71.

The terminal sections 41-43 of the short arm are very variable in expression (Figs. 17, 23) so as to make it difficult to establish homologies. However, when suitably matched chromosomes are chosen (Figs. 18, 22) it is clear that these segments are in fact homologous between the $2n$ and $3n$ larvae. The Ring of Balbiani is situated in the short arm in section 48 (Figs. 19, 23) and the banding pattern is disrupted in this region. The terminal bands in segment 53 are clearly identical with the "group of 5" in the base of IIL (59 distal) of *Prosimulium* species (1, 12). The bands in the remainder of this chromosome are homologous between $2n$ and $3n$ forms as shown for the short arm by Figs. 17, 19 vs. 23, 24 and for the long arm by Figs. 19–21 vs. 25.

Chromosome III

In the comparison of chromosome III in the diploids and triploids the homology of the band sequence was confirmed by careful slide examination. However, photographs of uniformly stretched chromosomes were difficult to obtain and as a result especially in the $3n$ forms the photographs incorporated are not adequate to demonstrate homology with the diploids throughout.

Chromosome III consists of 29 segments: the centromere is taken to be at 84 "C" (Figs. 28, 31), the short arm comprises 72–83 inclusive (Figs. 26–29, 31), and the long arm 84–100 (Figs. 28–32). The extreme end of the short

arm appears to be atypical when compared with the short arm of chromosome III of *Prosimulium* (12) and *C. dacotensis* and *C. erimites* (2), as there is no conspicuous "frazzle", though occasional indications of flaring ends are seen (Fig. 26). The "blister" area in 73 distal is always striking in triploid forms (Fig. 31) and occasionally in diploids (Fig. 28); the base of the "blister" (74 proximal) is formed by a heavily staining triple band (Figs. 26–28, 31). Just below this "blister" area, the sections 74–78, which are closely paired in $2n$ forms, fail to pair in the majority of the $3n$ larvae (Fig. 31). It is not clear to what extent non-homologies of constituents are responsible for this phenomenon. The segments in the long arm are homologous throughout, as seen in Figs. 28–32.

It is worth while pointing out that in comparison to *Prosimulium* (1, 12) the gross markers of five chromosome arms are preserved in "correct" association in *C. mutata** and that in addition five of the chromosome ends (all but IIIS) and one or more intercalary segments in each arm can be identified with *Prosimulium* sections of the same arm by their banding pattern. Thus it would seem that substantial interarm changes have not occurred in the evolutionary separation of *Prosimulium* and *Cnephia*.

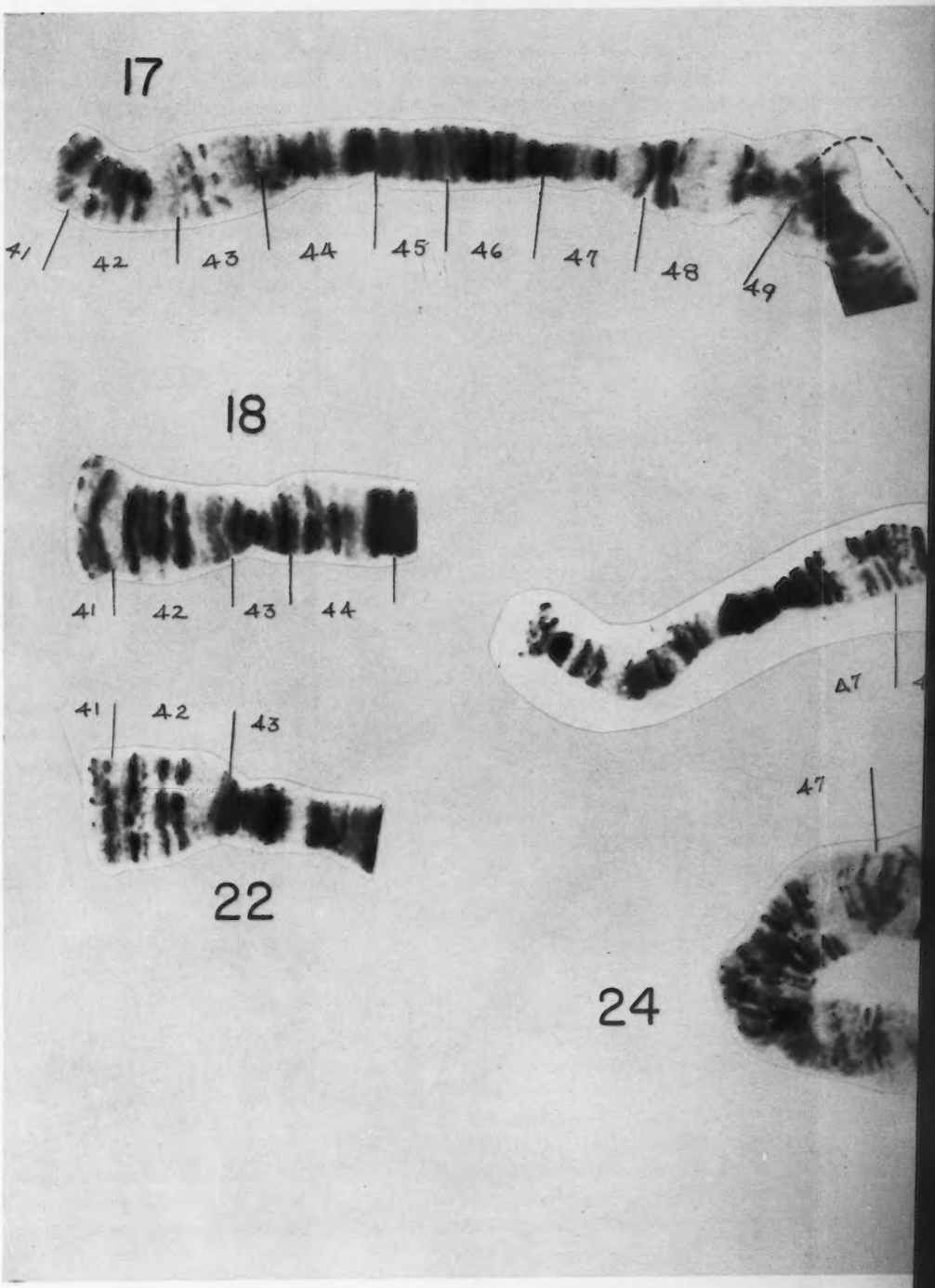
Structural Rearrangements

Chromosome Polymorphism in Diploid C. mutata

In diploid *C. mutata* females, no structural hybridity of any kind was detected. In contrast all 28 males were heterozygous for a rearrangement in the centromere region of the first chromosome which interfered with pairing throughout the expanded region (Fig. 7). It was not possible to determine in detail what structural changes accounted for this failure of pairing. All 54 females had completely paired centromeres in the first chromosome and the indications are that they were all homozygous for the identical sequence, namely that corresponding to the labelled constituent of Fig. 7. The restriction of structural hybridity to the male, the universal heterozygosity of males, and the absence of one kind of homozygote, all indicate a chromosomal sex determining mechanism of the XX–XY type with male heterogamety and conforming in all essential respects to that of other black fly species (1, 7, 12).

*IS base: "three heavy groups"; IL base: nucleolus; IIS: Ring of Balbiani; IIL: base "group of five"; IIIS: "blister".

Figs. 17–25. Photographic maps of chromosome II of diploid and triploid *C. mutata*. C: centromere; RB: Ring of Balbiani.
Figs. 17–19. IIS of diploid.
Figs. 19–21. IIL of diploid, showing also by brackets the break points of inversions IIL-1 and IIL-2 of the triploids.
Figs. 22–24. IIS of triploid.
Fig. 25. Entire chromosome II of triploid, heterozygous for inversion IIL-1 (SSt).
Figs. 26–32. Photographic maps of chromosome III of diploid and triploid *C. mutata*. C: centromere.
Figs. 26–30. Entire chromosome III of diploid.
Figs. 31–32. Entire chromosome III of triploid.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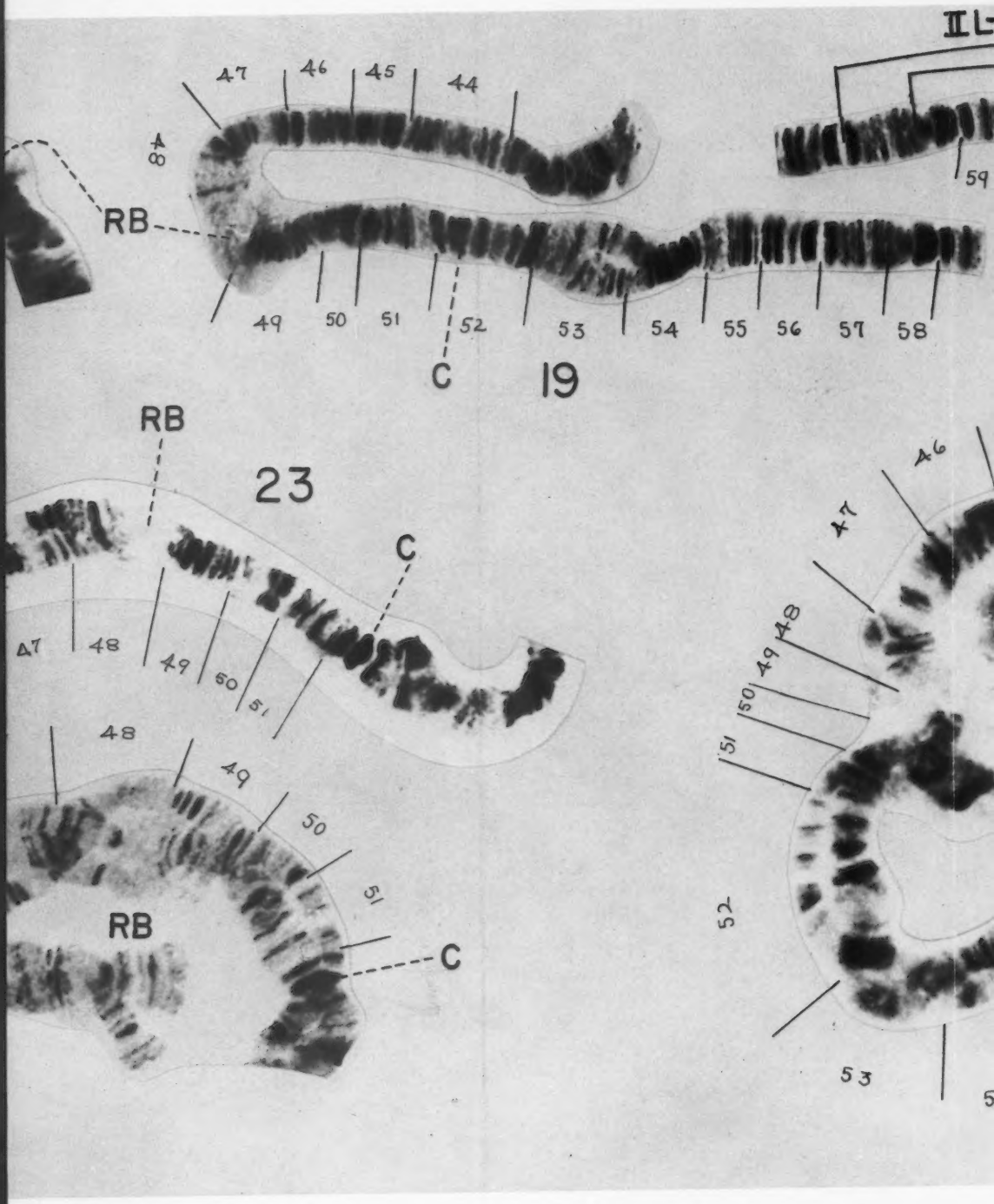
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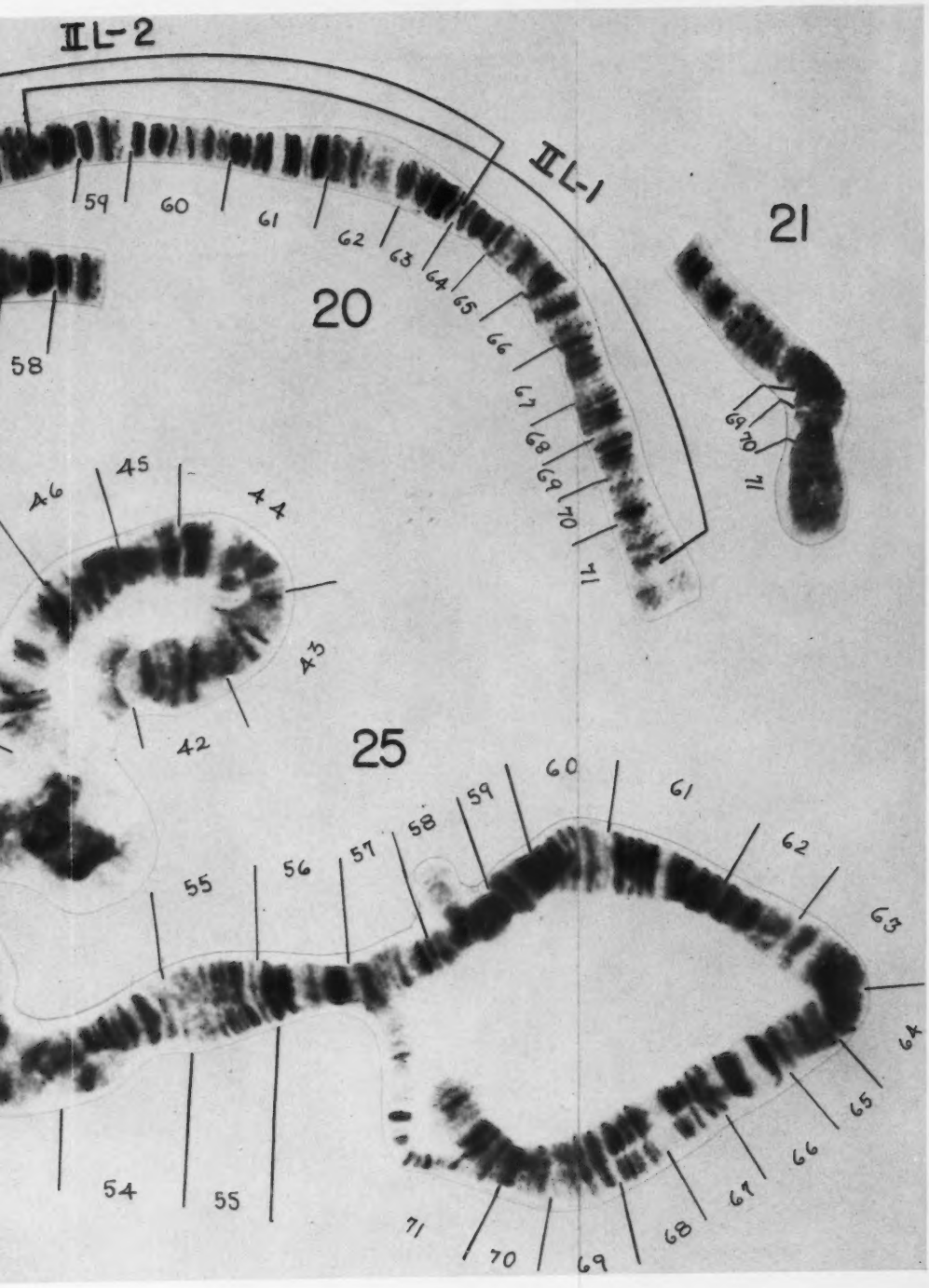
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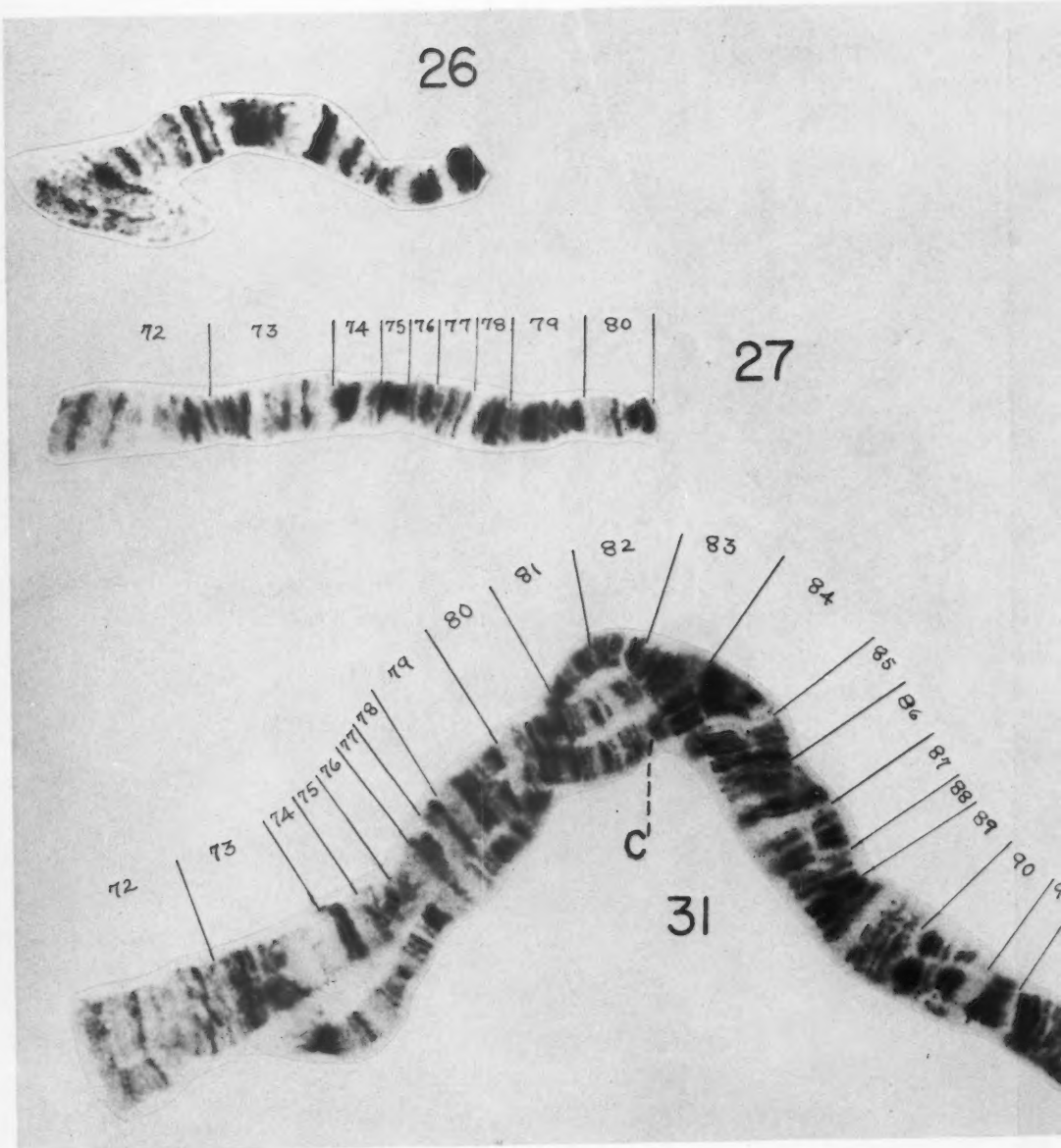
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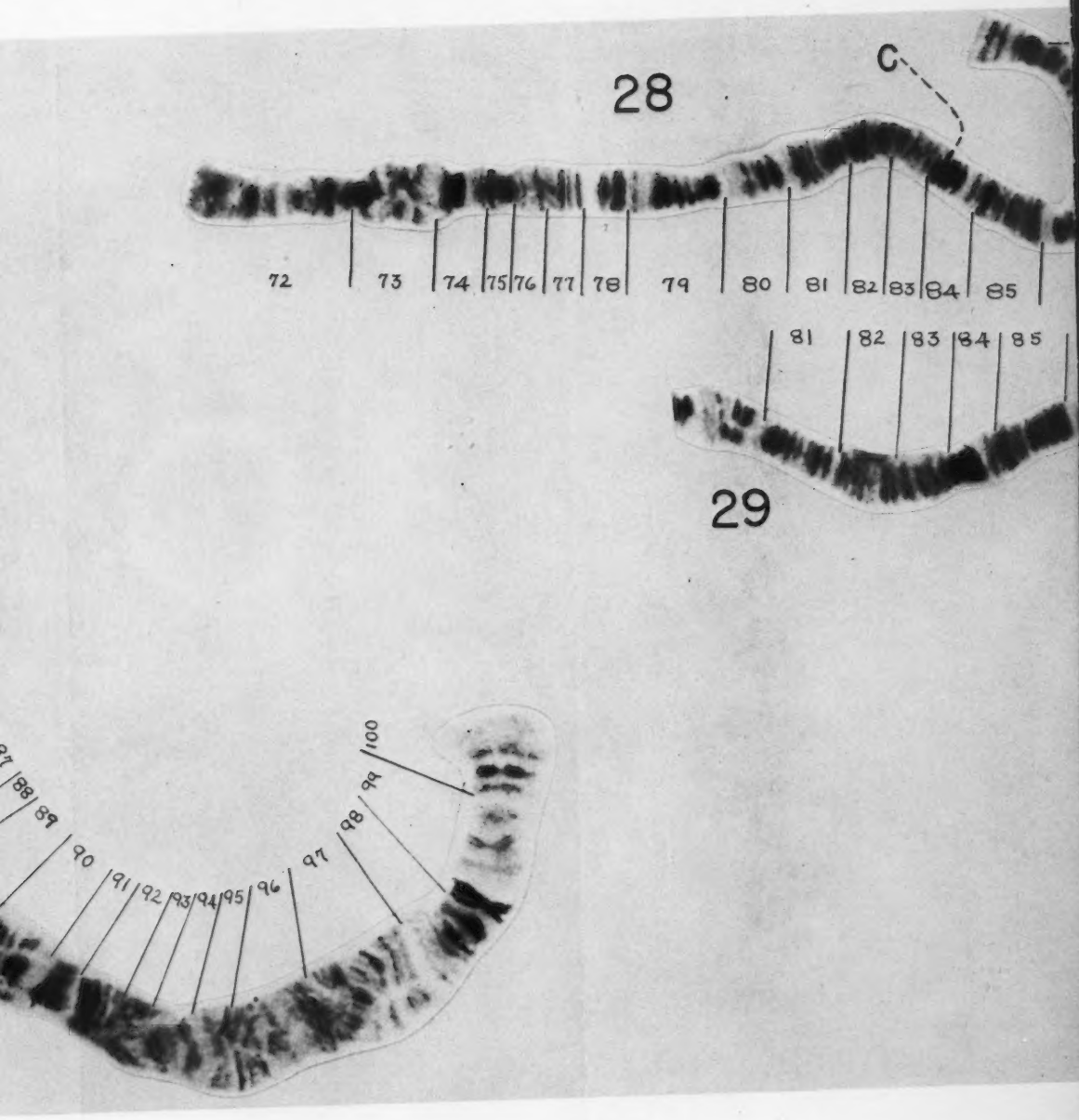
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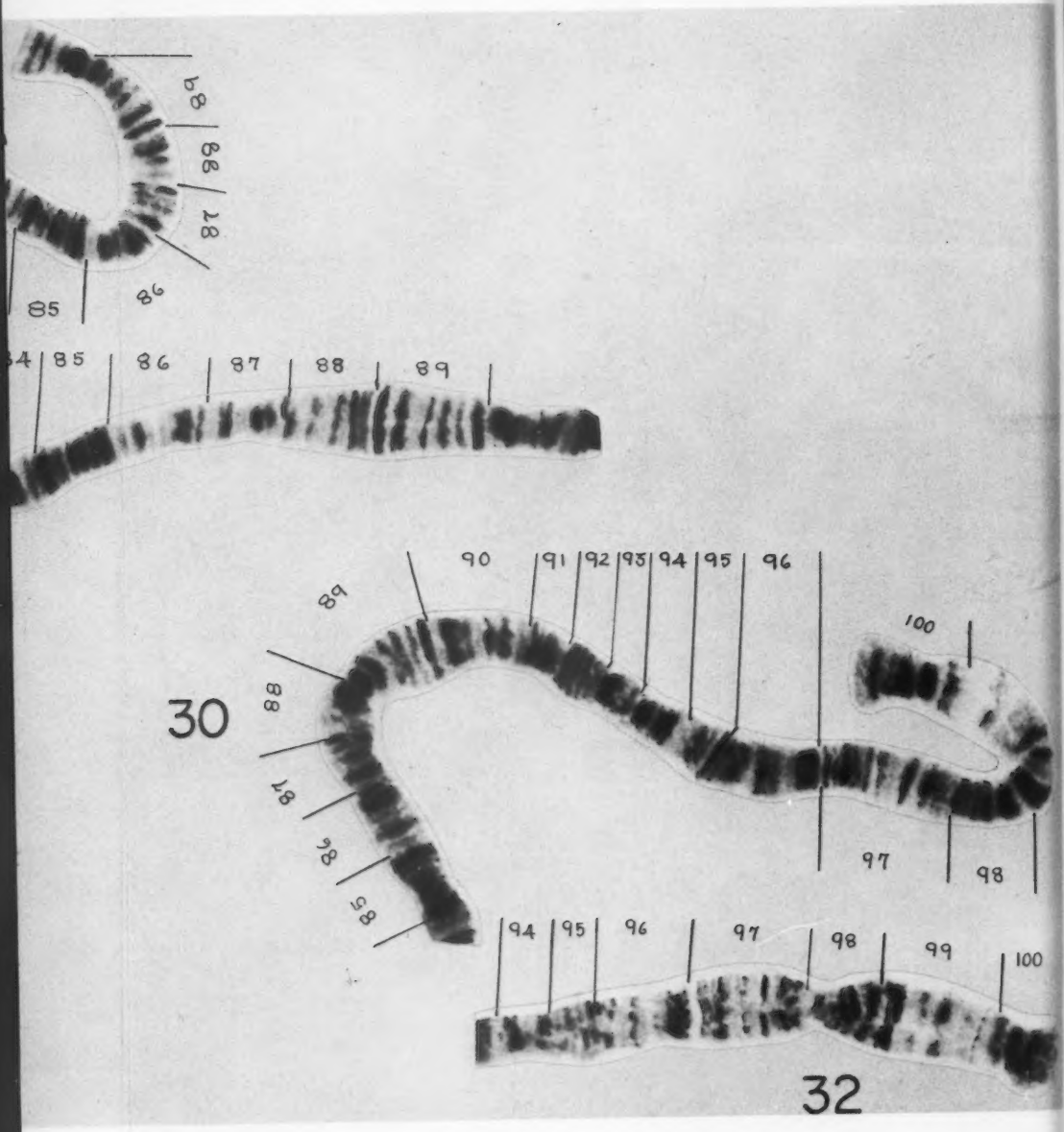
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Apart from the sex-associated rearrangement in centromere I no heterozygous sequences were seen in males. The complete absence of "autosomal" rearrangements in both sexes is remarkable and so far unique among black flies.

Chromosome Polymorphism in 3n

The structural rearrangements seen in *3n* larvae are simple inversions and no interchanges, deletions, or duplications were found.

There are four inversions in the nucleolar arm of chromosome I, and beginning from the end of the long arm they are numbered as IL-1, IL-2, IL-3, and IL-4. Two inversions were found in IIL; while IIL-1 is widely distributed, IIL-2 occurred only at Amable du Fond. Although there was some non-pairing in the third chromosome, this could not be interpreted as due to inversions.

Since in triploids each chromosome is represented by three homologues, individual larvae may be classified into one of four categories with respect to a given inversion. Two of these are homozygotes; standard (SSS) and inverted (III), and two are heterozygotes; two constituents having the standard sequence in one type (SSI) and two constituents having the inverted sequence in the second type (SII). The constitution of the Inglewood larvae with respect to inversions IL-1, IL-2, IL-3, and IIL-1 is shown in Table III. In the large inversion IIL-1 one expects to be able to differentiate all four possible combinations of sequences, and in fact all four were found. For each of the inversions in IL it should have been possible to distinguish alternative homozygous sequences, but only one kind was found and because of the small size of the inversions and the compactness of the loops the two kinds of heterozygotes could not be distinguished from each other and are tabulated jointly.

TABLE III

Constitution of 105 triploid *C. mutata* (Inglewood 1955–1957) with respect to IL-1, IL-2, IL-3, IIL-1

Constitution	IL-1	IL-2	IL-3	IIL-1
SSS	74	86	25	10
SSI	31	19	80	66
SII				10
III				19
	105	105	105	105

The approximate positions and dimensions of the floating inversions are indicated in the idiograms (Fig. 2) and the exact break points for each of the inversions described below are shown on the diploid standard maps by brackets.

IL-1.—This is a simple inversion close to the end of the long arm with one break point in the almost clear gap between segments 35 and 36, and the other in the distal third of 39 (Fig. 12). In the heterozygous condition (Fig. 13) it is expressed as a small, completely paired loop and in the majority

of the slides it is entangled and difficult to study. Of the 105 Inglewood larvae examined 31 were heterozygous and of the 10 that could be traced through all were of the "SSI" type. The same inversion was also found in one larva from Amable du Fond where it was associated with IL-3.

IL-2.—This inversion is somewhat smaller than IL-1 and extends from the center of segment 33 to the section limit 34/35 (Fig. 11). The heterozygotes rarely form completely paired loops and are very often characterized by non-pairing of the strands. At Inglewood this inversion was recovered only in the 1957 collections. It was not found in the small sample from Amable du Fond.

IL-3.—Over the 3-year collecting period at Inglewood this was the commonest heterozygous inversion. It occupies the region close to the nucleolar organizer with break points at the section limits 26/27 and 28/29 (Fig. 11). IL-3 also occurred at Amable du Fond, both alone and with IL-1.

IL-4.—This inversion occurred exclusively in larvae from the Kahshe area where it is involved in a complex heterozygous configuration. Simple heterozygotes for IL-4 were not found nor was the homozygous sequence IL-4 seen. An analysis of the heterozygotes in Kahshe individuals showed (Figs. 16*a*, 16*b*) that there is strong pairing in three rearranged segments labelled BC, DE, and FG in Fig. 16*b*. The large central pairing segment DE has identical orientation in all constituents and corresponds to the inversion IL-3 (Inglewood) carried in one constituent; the two peripheral pairing regions are reversed in orientation between constituents and delimit inversion IL-4 (Kahshe) carried in the other two constituents. The break points of IL-4 are in the centers of segments 26 and 30 (Fig. 11) and this inversion is seen to include the Inglewood inversion IL-3 on the map. Assuming that simultaneous four-break rearrangements do not occur, it is clear that IL-3 and IL-4 must be phylogenetically related through the standard sequence.

IIL-1.—This inversion takes in a large part of the chromosome arm. It extends from segment 58 proximal to 71 distal, leaving only a few light terminal bands in place (Fig. 25). The break points are shown in Fig. 20. Because of its common occurrence and the distinctive loop formed in heterozygotes this inversion is very useful for the identification of *C. mutata* (triploids) and for analytical work on inversion associations (Table III, Fig. 34). This inversion was common at Inglewood; it also occurred in larvae from Etobicoke Creek, Victoria, Peel County, Ontario, and at Long Lake (Eaton Pond outlet), Hamilton County, N.Y. It was found in three of the six larvae from Amable du Fond in association with both IL-1 and IL-3.

IIL-2.—This inversion was observed only in two larvae from Amable du Fond which were not otherwise heterozygous. As indicated by the brackets in Fig. 20, IIL-2 overlaps IIL-1 on the map.

Association of Inversions

In a bisexual species with random mating one would expect chromosome rearrangements to be randomly assorted in individuals of a population, and selective mortality or abnormal mating pattern would be reflected in deviations

from the equilibrium condition. On the other hand inversions in parthenogenitically reproducing organisms should not be randomly assorted since the various clones in which they originate are kept apart by the absence of cross-fertilization. An analysis of the association of inversions in triploid *C. mutata* was therefore undertaken to see whether any information could be extracted regarding the origin of triploidy and the evolution of chromosomal polymorphism in the triploids. In particular it was expected that inversions of

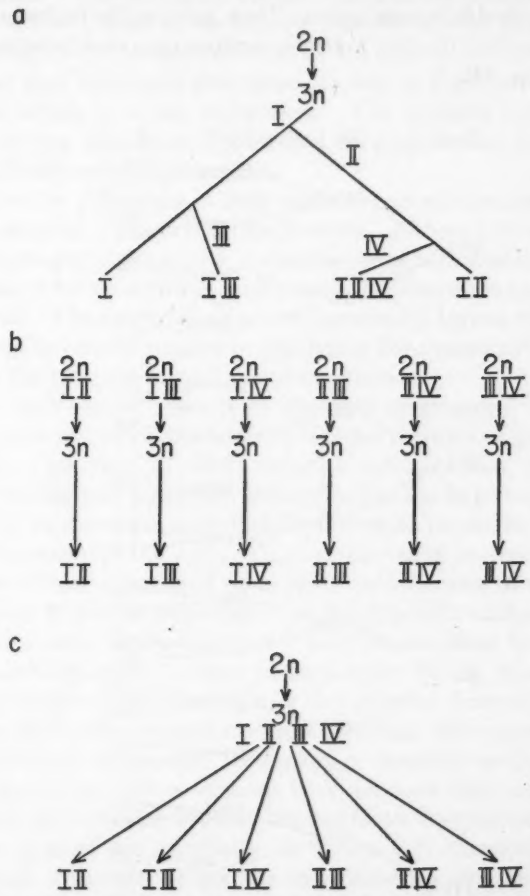

FIG. 33*a*. Pattern of inversion associations expected in an obligate parthenogenetic form. Roman numerals denote heterozygous inversions. If inversions (such as III and IV) originate in a parthenogenetic form, they are restricted in their association to inversions already carried in the same chromosome. Certain associations (those involving II–III and III–IV) are "forbidden".

FIG. 33*b*. Pattern of inversion associations actually observed and its interpretation if parthenogenesis is apomictic. Only the various double heterozygotes are shown.

FIG. 33*c*. Pattern of inversion associations actually observed and its interpretation if parthenogenesis is automictic. Only the double heterozygotes are shown.

recent origin would be restricted in their association to those that occurred earlier in the same clone so that within clones it should be possible to deduce the order in which inversions arose (Fig. 33*a*). In addition inversions occurring in separate clones should be mutually exclusive in individual larvae. However, in the relatively small sample of 105 larvae from Inglewood, of the 32 possible sequence combinations (pooling the two kinds of heterozygotes for chromosome I inversions) 15 were actually found (Fig. 34) and these include all six possible double heterozygotes. Thus, as far as the Inglewood population is concerned each of the four known inversions can occur heterozygously with any other (Figs. 33*b*, *c*).

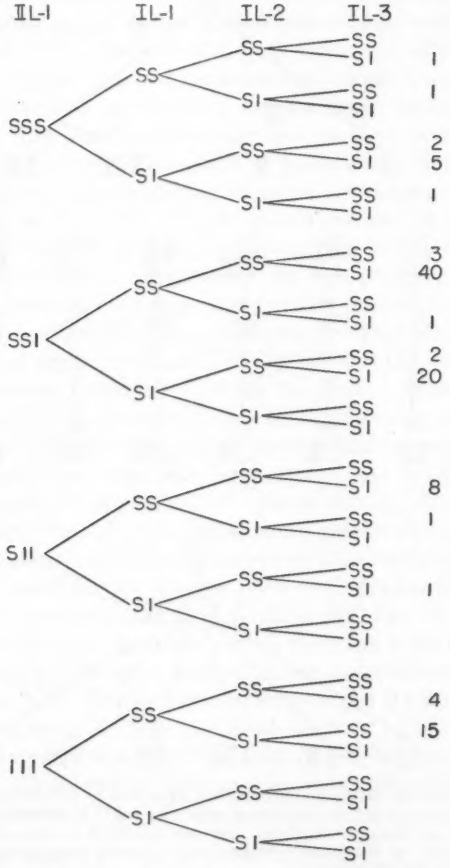

FIG. 34. Association of arrangements in IL and IIL in 105 triploid *C. mutata* from Inglewood. The two kinds of heterozygotes (SSI and SII) for first chromosome inversions are pooled and represented as SI.

Discussion

Reproductive Isolation of 2n and 3n Forms

Study of the salivary gland chromosomes has shown that in each of the six chromosome arms one band sequence exists which is common to the *2n* and *3n* larvae and which was therefore chosen as standard for that arm; the possibility of minor structural differences especially in heterochromatic regions is not entirely excluded. The sex ratio, diploid chromosome number, and well-differentiated *XX–XY* mechanism indicate that the diploid forms are bisexually reproducing. No evidence was found for the existence of parthenogenetic *2n* females, but their occasional occurrence can only be ruled out by extensive breeding tests, which were not undertaken. The triploids include females only, no males having been found in more than 300 sexed larvae; they reproduce entirely by thelytokous parthenogenesis.

In contrast to the differences in their mode of reproduction the two forms follow similar seasonal cycles in their life histories. Although by and large the diploids develop earlier in the season, mature larvae of both siblings were found at the same time in the same stream, and presumably the adults are on the wing at the same time. The abnormal sex ratios reported by Davies (6) from adult emergence cages in various streams in Algonquin Park presumably find their explanation in the presence of such mixed populations.

Since in the area studied there is no apparent geographical or ecological barrier between the two forms, one enquires whether there is any gene exchange between them. One piece of direct evidence indicates that there is not. Heterozygous "autosomal" inversions are confined to the *3n* larvae where they are common; all *2n* forms are completely homozygous except for the heterozygous sex arrangement in the male. This non-sharing of inversions indicates that there is no effective means of exchanging chromosomes or chromosome segments between *2n* and *3n* individuals. A priori, genetic exchange between diploids and triploids is highly improbable since meiosis must be profoundly modified in parthenogenetic females to perpetuate the *3n* condition. The postulate for consistently balanced (*2n* or *3n*) progeny from cross matings entails complex assumptions regarding effects of mating and presence of sperm on oöcyte development in females. Tetraploid or aneuploid progeny is much more likely to result but such individuals have not been observed. Thus we conclude that effective crossing between the two forms does not take place.*

As has been pointed out especially by White (25) taxonomic usage is awkward in such situations. Since the two forms do not interbreed, the diploids may be said to constitute a biologically distinct species of their own. As to the triploids the species concept is not readily adapted to parthenogenetic forms where cross-fertilization has been completely abandoned and numerous independent clones exist.

*All these a priori considerations to the contrary, we have recently obtained samples of a new triploid species of *Prosimulium* from the vicinity of North Bay, Ontario, in which triploid males occur in numbers. The origin and function of these males remain to be determined.

Mode of Parthenogenetic Reproduction

Parthenogenesis which results exclusively in female offspring is usually classified into two main types (25): (1) ameiotic or apomictic parthenogenesis where the maturation division is mitotic in character, and where all the progeny are genetically like the parent, barring new mutations and rearrangements; and (2) meiotic or automictic parthenogenesis where a regular meiosis takes place and where, depending upon the mode of compensatory chromosome doubling, genetic and cytological (inversion) segregation may occur in the progeny.

Several kinds of evidence appear to indicate that parthenogenesis in *C. mutata* is meiotic. First, the independent inversions 1 and 2 in the long arm of chromosome I (IL-1 and IL-2) and the corresponding standard sequences were found assorted in all four possible combinations: S/S, 1/S, S/2, 1/2, in individual constituents of the triploid females. Thus, in the constituents analyzed S/S was found at least 168 times; 1/S, 30 times; S/2, 18 times; and 1/2, 2 times. Unless triploidy originated repeatedly in a diploid population in which all four types of chromosomes already existed, reassortment of inversions by crossing over is indicated.

Second, with regard to inversion IIL-1 it is seen (Table III) that all four possible classes of larvae (SSS, SSI, SII, III) were actually encountered among the triploids. Again, unless triploidy originated repeatedly in a diploid population in which arrangement IIL-1 existed homozygously, heterozygously, and "azygously" the existence of these four classes of larvae in *3n* forms can only be accounted for by the occurrence of crossing over in meiosis. Finally, unless parthenogenesis in *C. mutata* is meiotic the 105 Inglewood larvae (Fig. 34) must represent at least 15 different triploid clones, each separately derived from a highly heterozygous diploid progenitor population. The number of such separate clones goes up still further as other inversions are found which "assort freely" with those from Inglewood. In short, if we postulate apomixis we must accept the corollary that triploidy in *C. mutata* originated independently in many different lines (Fig. 33*b*), which were favored by mass selection over their diploid forebears and most of which survived to this day.* Contrariwise, if we postulate meiotic parthenogenesis, the number of independent lines "required" is much reduced, e.g. all Inglewood lines can be derived by segregation from a single triploid individual if this is heterozygous for the four inversions (Fig. 33*c*). Although apomixis is not strictly excluded, it would seem that the inversion associations observed in present-day lines are more readily understood on the assumption that crossing over does occur. The further discussion therefore accepts the notion that parthenogenesis in *C. mutata* is meiotic.

Among the parthenogenetically reproducing plants and animals meiotic parthenogenesis is relatively rare (8, 23, 25) and the possibility of its occurrence in triploids has been discounted by most authors (23, p. 234; 25, p. 360).

*Stalker in a recent publication (Stalker, H. D. On the evolution of parthenogenesis in *Lonchoptera* (Diptera). Evolution, 10, 345–359 (1956).) has presented evidence that parthenogenesis in *Lonchoptera dubia* Curran evolved gradually and in many semi-isolated small populations. The cytological indications are that parthenogenesis is automictic in this form.

However, Stalker (19) has postulated that automictic parthenogenesis may be the mode of reproduction in the entirely triploid and exclusively female populations of the fly *Ochthiphila polystigma* (Chamaemyidae) albeit without genetic or cytological evidence that meiosis actually occurs in this species.

Of the various kinds of automictic parthenogenesis (25), that involving a doubling of the chromosomes in the oögonial division followed by a normal meiosis, as in parthenogenetic earthworms and planarians, seems excluded for *C. mutata* since doubling of chromosomes was not observed in a study of oögonial divisions. A fusion of reduced sister cleavage nuclei as in certain Lepidoptera is improbable as a mechanism in a triploid where the products of meiosis would be unbalanced to begin with and where heterozygosis is common. Thus it would seem that the triploids must resort to a modified meiosis. Two such modifications are known which might work in an anisopolyploid ($3n$, $5n$...). Following pairing and crossing over, the somatic chromosome number may be reconstituted if the first division is abortive and the second division is a simple mitosis (Fig. 35*a*). One of the resulting nuclei may function as the egg nucleus, the other being extruded as a polar body. This in principle is the course of events in at least some diploid parthenogenetic *Artemia* (Crustaceae) (25) and in tetraploid parthenogenetic *Solenobia lichenella* (Lepidoptera) (11), as well as in "apomictic" triploid *Taraxacum* (Compositae) if the interpretation of Darlington (5) is accepted. First division restitution also occurs in the thelytokous wasp *Nemeritis canescens* (17) and a modification of the same basic scheme is found in the psychid moth *Apterona helix* (10) where restitution of the somatic chromosome number is brought about by a fusion of the second metaphase spindles, and where both resulting nuclei function in embryogeny.

Alternatively one may postulate that meiosis is complete and results in four reduced and generally aneuploid nuclei which are linearly arrayed across the egg. The normal triploid somatic complement may then be reconstituted if there is regularly a fusion between the two complementary central nuclei (Fig. 35*b*). This has been reported for various diploid parthenogenetic coccids of the genus *Lecanium* (e.g. 22) and automictic fusions are also known in several species of *Drosophila* (18). In particular Stalker (18) has presented strong genetic evidence that fusion between two or three reduced nuclei accounts for the production of occasional impaternate diploid and triploid progeny by virgin females of *D. parthenogenetica*.* While the fusion pattern is variable in *D. parthenogenetica* and involves the two central nuclei only about 50% of the time, the fusion pattern in the parthenogenetic Acalypterate *Lonchoptera dubia*, according to Stalker (19), is fixed and normally restricted to the two median nuclei. With this type of evolutionary restriction in the nuclear fusion pattern, only balanced types would result and anisopolyploids could perpetuate themselves efficiently.

*The fusion pattern postulated by Stalker has recently been demonstrated in cytological studies of his student Le Etta Sanger (Stalker, personal communication).

The genetic effects of these two schemes—abortive first division (Fig. 35*a*), and automictic fusion of complementary nuclei (Fig. 35*b*)—are indistinguishable, since both involve refusion of meiotic products in such a way as to reverse the effects of the first meiotic division. On either scheme, without crossing over between the centromere and a heterozygous inversion marker

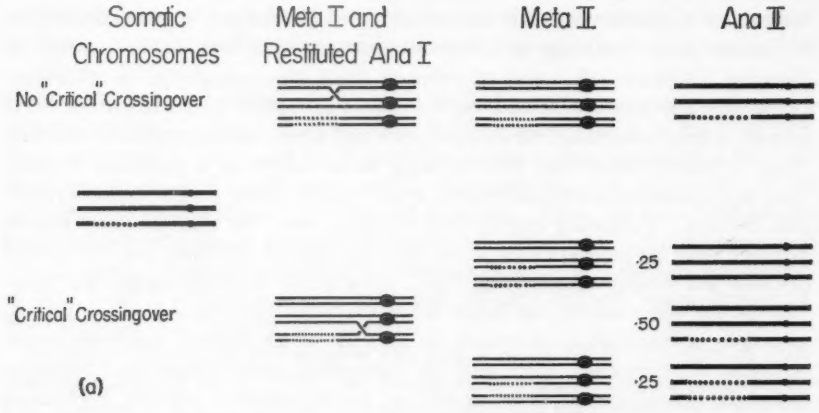

FIG. 35*a*. The genetic consequences of first-division restitution in oöcytes of a parthenogenetic triploid.

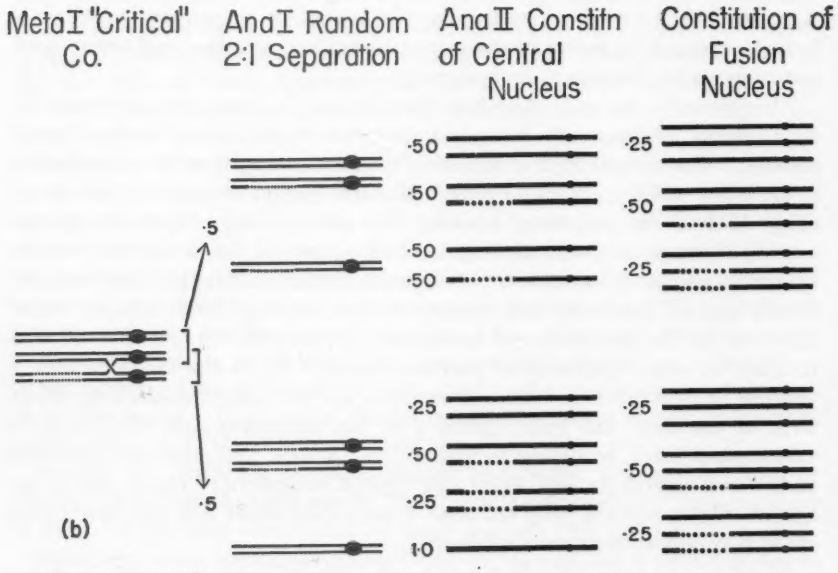

FIG. 35*b*. The genetic consequences of fusion of the central nuclei following completion of meiosis in oöcytes of a parthenogenetic triploid.

(or with even numbers of exchange per chromatid), all progeny will be like the parent. With odd numbers of exchanges, 50% of the progeny will be like the parent, 25% will have the frequency of the parental "simplex" constituent raised from one to two, and 25% of the progeny will have lost the "simplex" arrangement altogether. Continued operation of this process should lead to rapid depletion of heterozygosis and the eventual elimination of hybridity. However, in all investigated populations (e.g. Fig. 34) heterozygotes predominate and it is therefore clear that loss of heterozygosis by crossing over must be balanced by greater fitness or higher reproductive rates of heterozygous individuals. How great this heterotic advantage is we cannot tell unless an independent estimate of the frequency of critical crossing over is obtained.

Origin of Parthenogenesis and Triploidy in C. mutata

Many discussions have been published on the origin of polyploid parthenogenetic forms. The main issues: parthenogenesis first versus polyploidy first, apomictic versus automictic inception of parthenogenesis, and allopolyploidy versus autopolyploidy have all been argued. As far as the origin of anisopolyploids is concerned two rather distinct evolutionary pathways emerge as possibilities. The first of these proposed by Suomalainen (23) and supported by White (25) visualizes an origin of thelytokous (and apomictic) triploids by hybridization between an apomictic diploid form and a related bisexual species (or possibly between a bisexual diploid and a related tetraploid apomict). The second, sponsored by Stalker (19), proposes that a bisexual ancestor first gave rise to an automictic unisexual diploid population which in turn produced triploid automicts through a modification and extension of the fusion pattern of the reduced nuclei. As a final evolutionary possibility all vestiges of meiosis may be lost to give a truly apomictic triploid. The intervention of an automictic diploid, while possible, does not seem a necessary feature of such a scheme, but in any case hybridization is not involved.

The demonstrated existence of a structurally homozygous bisexual diploid form of *C. mutata* and the fact that in triploids the various heterozygous inversions occur in many combinations (Fig. 34) might, on the face of it, suggest a multiple and allopolyploid origin of triploidy (Fig. 33*b*), *2n C. mutata* contributing the standard sequence and a related hypothetical diploid apomict the various inverted sequences. Against this interpretation is the absence of diploid parthenogenetic forms not only in *C. mutata* but also among the relatives of other triploid black fly species and in black flies in general.* Furthermore, if we are correct in inferring automixis in *C. mutata* then any interpretation of the "allopolyploid type" is excluded for it can hardly be supposed that a diploid apomict when crossed with a bisexual form yielded a reproductively competent triploid automict.

On the other hand if we favor an autopolyploid and automictic origin of *3n C. mutata* we must account for the maintenance of hybridity and for the

*Mr. J. A. Downes (Department of Agriculture, Ottawa) has correctly pointed out to us that facultative apomicts in black flies would not be readily detected by current procedures.

resemblance of inversion association to those of bisexual species. If all triploid *C. mutata* trace back to a single triploid ancestor we may postulate that this was heterozygous for a large number of inversions which by meiotic parthenogenesis became sorted out into the diverse associations observed in present day lines (Fig. 33*c*) or that in the propagation of triploids by chance or in consequence of selections effects new inversions arose or became established mainly in individuals heterozygous for "older" inversions. Alternately, if triploidy in *C. mutata* is polyphyletic one has only to postulate a highly developed chromosomal polymorphism in the diploid progenitor population. In either case automixis and maintenance of chromosome hybridity can be reconciled if heterosis is invoked.

In conclusion all available information can be understood in terms of two alternative interpretations. (1) Triploid *C. mutata* are of allopolyploid and highly "polyphyletic" origin and are perpetuated by ameiotic (apomictic) parthenogenesis, and (2) triploid *C. mutata* are autopolyploid and not necessarily "polyphyletic" in origin; automixis was their mode of parthenogenetic reproduction at least in the beginning, and loss of heterozygosis by crossing over is balanced by heterosis of individuals carrying inversions. The former interpretation is not excluded, the latter is preferred. Final proof of meiotic parthenogenesis required the demonstration of chromosome segregation in the progeny of structurally hybrid triploid females. This seems a more promising approach than direct studies on meiosis, which are difficult in dipterous females.

Whatever may have been the course of evolution of triploid parthenogenesis in *C. mutata*, it is evident that it has been exploited repeatedly and successfully by several unrelated black fly species. The situation in northern *Prosimulium ursinum*, *P. macropyga*, and *Gymnopais* sp. is strictly comparable to that in *C. mutata*, except that no diploids have ever been found. All individuals are triploid and female, and all are highly heterozygous (unpublished data). It may be postulated that they too reproduce by meiotic parthenogenesis and that a modicum of recombination within individuals combined with high reproductive capacity (all female progeny) may compensate for the loss of the ancient cross-fertilizing system and enable triploids to compete successfully in some environments. The northern distribution of triploids suggest that this occurs more frequently at the extremes of the range of the diploid progenitors, just as has been proposed for the triploid form (coelebs) of the isopod *Trichoniscus elizabethae* (24) and is generally recognized to be valid for plants (4).

Summary

Diploid and triploid individuals occur together in larval populations of *C. mutata* in southern Ontario. The diploids have a normal sex ratio and an *XX–XY* type sex determining mechanism with male heterogamety and differential segments in the centromere region of the first chromosome. All triploids are female; presumably they reproduce by thelytokous parthenogenesis.

The banding pattern of the salivary gland chromosomes was compared in diploids and triploids and the identical "standard" sequence was found in both. Whereas the triploids exhibit pronounced inversion polymorphism no "autosomal" heterozygous rearrangements were detected in the diploids. This restriction of inversions indicates that effective crossing between diploids and triploids does not occur and that these forms constitute reproductively isolated and biologically distinct species.

Evidence is presented that parthenogenesis in the triploids is meiotic and automictic, and that hybridization is probably not involved in the evolution of anisopolyploid parthenogenetic forms in black flies.

References

1. BASRUR, P. K. The salivary gland chromosomes of seven segregates of *Prosimulium* (Diptera: Simuliidae) with transformed centromere. Can. J. Zool. **37**, 527–570 (1959)
2. BASRUR, V. R. Cytotaxonomic studies in black flies and midges. Ph.D. Thesis, University of Toronto, Toronto, Ontario. 1957.
3. CONGER, A. D. and FAIRCHILD, L. M. A quick-freeze method for making smear slides permanent. Stain Technol. **28**, 281–283 (1953).
4. DARLINGTON, C. D. Chromosome botany. Allen and Unwin, London. 1956.
5. DARLINGTON, C. D. and MATHER, K. Elements of genetics. Allen and Unwin, London. 1950.
6. DAVIES, D. M. A study of the black fly population of a stream in Algonquin Park, Ontario. Trans. Roy Can. Inst. **28**, 121–160 (1950).
7. DUNBAR, R. W. The salivary gland chromosomes of seven forms of black flies included in *Eusimulium aureum* Fries. Can. J. Zool. **37**, 495–525 (1959).
8. GUSTAFFSON, A. Apomixis in the higher plants. I. The mechanism of apomixis. Lunds Univ. Årsskr. N.F. Avd. 2, **42** (2), 1–66 (1946).
9. HOCKING, B. and RICHARDS, W. R. Biology and control of Labrador black flies (Diptera, Simuliidae). Bull. Entomol. Research, **43**, 237–257 (1952).
10. NARBEL, M. La cytologie de la parthénogénèse chez *Apterona helix* Sieb. (Lepidoptera: Psychides). Rev. suisse zool. **53**, 625–681 (1946).
11. NARBEL-HOFSTETTER, M. La cytologie de la parthénogénèse chez *Solenobia*s p. (*lichenella* L.?) (Lepidopteres, Psychides). Chromosoma, **4**, 56–90 (1950).
12. ROTHFELS, K. H. Black flies: siblings, sex, and species grouping. J. Heredity, **47**, 113–122 (1956).
13. ROTHFELS, K. H. and DUNBAR, R. W. The salivary gland chromosomes of the black fly *Simulium vittatum* Zett. Can. J. Zool. **31**, 226–241 (1953).
14. SHEWELL, G. E. Interim report on distributions of the black flies (Simuliidae) obtained in the Northern Insect Survey. Canada Dept. Natl. Defence, Defence Research Bd. Envir. Prot. Tech. Rept. No. 7 (1957).
15. SOMMERMAN, K. M. Identification of Alaskan black fly larvae. Proc. Entomol. Soc. Wash. **55**, 258–273 (1953).
16. SOMMERMAN, K. M., SAILER, R. I., and ESSELBAUGH, C. O. Biology of Alaskan black flies (Simuliidae, Diptera). Ecological Monographs, **25**, 345–385 (1955).
17. SPEICHER, B. R. Oogenesis in a thelytokous wasp, *Nemeritis canescens*. J. Morphol. **61**, 453–471 (1937).
18. STALKER, H. D. Parthenogenesis in *Drosophila*. Genetics, **39**, 4–34 (1954).
19. STALKER, H. D. A case of polyploidy in Diptera. Proc. Natl. Acad. Sci. **42**, 194–199 (1956).
20. STONE, A. The Simuliidae of Alaska. Proc. Entomol. Soc. Wash. **54**, 69–96 (1952).
21. STONE, A. and JAMNBACK, H. A. The black flies of New York State (Diptera: Simuliidae). N.Y. State Museum Bull. 349 (1955).
22. SUOMALAINEN, E. Beiträge zur Zytologie der parthenogenetischen Insecten. II. *Lecanium hemisphaericum* (Coccidae). Ann. Acad. Sci. Fennicae, A, **57**, 1–30 (1940).
23. SUOMALAINEN, E. Parthenogenesis in animals. Advances in Genet. **3**, 193–253 (1950).
24. VANDEL, A. La parthénogénèse geographique. IV. Polyploidie et distribution géographique. Bull. biol. **74**, 94–100 (1940).
25. WHITE, M. J. D. Animal cytology and evolution. Cambridge University Press, London. 1954.

LOCOMOTOR PERFORMANCE OF CHUM SALMON FRY (ONCORHYNCHUS KETA) DURING OSMOREGULATORY ADAPTATION TO SEA WATER[1]

ARTHUR HILLIER HOUSTON[2]

Abstract

Transfer into sea water produced an immediate and marked depression of the cruising speed of chum salmon fry. Despite considerable recovery from this initial effect the fish exhibited a continuing slight depression of cruising speed over the entire experimental period (80 hours). Variations in total body levels of chloride and water were significantly correlated with changes in cruising speed, suggesting that variations in activity might be related to the process of osmoregulatory adaptation to sea water. Possible mechanisms underlying this effect have been discussed.

Introduction

Juvenile Pacific salmonids have the ability to survive direct transfer into sea water at certain stages of their life cycles (2, 17). Nevertheless, even successful adaptation to sea water is accompanied by an initial adjustive phase during which the fish must tolerate marked departures from regulated water–electrolyte balance. Characteristic of such variations are the raised levels of plasma, tissue, and cellular electrolytes; loss of cellular fluids; and variations in plasma–electrolyte ratios described for steelhead trout (17) and by Black (2) for chum salmon fry.

Increased energy demands for osmoregulation during this phase of adaptation, together with the possible inhibition of electrolyte-sensitive components of the neuromuscular apparatus might be expected, a priori, to influence the activity of the animals. Indeed, several authors including Huntsman and Hoar (18), Shepard (23), Houston (16), and Hoar (1958, unpublished data) have observed decreases in the activity of salmonids subjected to abrupt increases in environmental salinity.

The investigation reported herein has been carried out in an attempt to describe in a quantitative manner the effect of transfer into sea water on the locomotor performance of one species of Pacific salmon, the chum (*Oncorhynchus keta*). Determinations have been made of the cruising speeds of individual fish after various periods in sea water. Variations in total body concentrations of chloride and water were determined as a measure of the general course of osmoregulatory adaptation to sea water, and the correlation between stage of adaptation and relative activity determined.

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Contribution of the Department of Zoology, and Institute of Fisheries, The University of British Columbia, with financial assistance from the National Research Council of Canada. A portion of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2]Present address: Zoological Laboratory, Dalhousie University, Halifax, N.S.

Materials and Methods

A. Materials

Chum salmon fry used in these experiments were obtained from the Pacific Biological Station, Nanaimo, British Columbia, through the courtesy of Dr. Ferris Neave. The fish were maintained in running dechlorinated Vancouver city water at the University hatchery, and were fed a dry commercial diet[3] supplemented with yeast and cod liver oil. Growth was good, and following the "delayed mortality" commonly encountered after transport of salmonids, there were few deaths in the experimental stocks during the course of the investigation. Water temperatures varied from 10 to 16° C over the course of the summer. No attempt was made to control light intensity or day length, and the fish were subjected to normal spring and summer light conditions in the hatchery.

B. Methods

(*1*) *Determination of Cruising Speeds*

(*a*) *Apparatus*

An artificial stream constructed by Mr. T. G. Northcote of the British Columbia Game Commission was modified for use in the determination of cruising speeds. The general construction of this apparatus is indicated in Fig. 1. Water temperature was maintained at 10.0 ± 0.5° C, and light at 0.8 foot-candles (measured at the water surface). An overhead arrangement of lights and mirrors gave even surface illumination, and allowed observation of the fish with a minimum of disturbance.

An inset channel (6, in Fig. 1) measuring 56 by 20 by 5 cm and screened at both ends with coarse-mesh plastic screening served as the experimental channel.

[3]Supplied through the courtesy of Mr. J. R. Clarke, Salt Lake City, Utah.

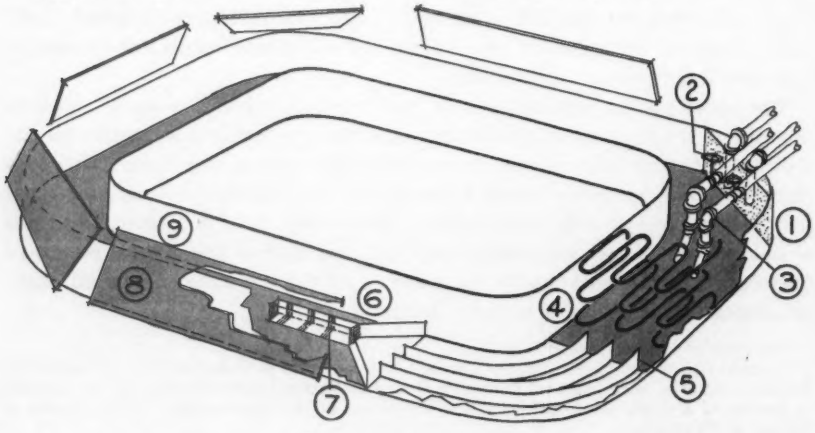

Fig. 1. Apparatus used in determination of cruising speed: (1) water intake pipes, (2) control valves, (3) water outflow pipes, (4) refrigeration unit, (5) turning vanes, (6) performance channel, (7) rheotactic cue lines, (8) mirrors, (9) lights.

Visual cues for rheotactic response consisted of vertical and horizontal bars painted down the sides and across the floor of the channel. The balance of the visual field of the fish and the remainder of the apparatus was painted grey.

The two ½ horse power circulating pumps were operated at constant rate, and water velocity regulated by means of valves on the outflow pipes (2, in Fig. 1). Current velocities were determined by means of a Steven midget current meter.[4]

(*b*) *Procedure*

Fish were acclimated to sea water (salinity, 22–24‰) in 5-gal. all-glass aquaria at culture-trough water temperatures. The acclimation times were such that, allowing three and a half to four hours for each determination, the total durations of the acclimation periods fell into the ranges: 3½–4, 9–10, 13–14, 20–22, 34–36, 48–54, and 76–82 hours.

The apparatus was filled with sea water (salinity, 22–24‰) to a depth of 4 cm. Single fish were transferred in water from the acclimation aquaria to the performance channel. A period of 1 hour was allowed for recovery from any inadvertent shock of handling. During this interval water velocity was held at a level just sufficient to ensure adequate temperature control (2–3 cm/sec), and evoked only mild and transitory positive rheotactic responses.

Following this period water velocity was increased by increments of 1–2 cm/sec every 5 minutes. At velocities of 6–10 cm/sec strong positive rheotactic responses were invoked in fish of good condition. Individuals failing to respond within this current range were discarded. In all, 7 fish of the 98 tested fell into this category. Water velocities were further increased until the fish were swept back against the downstream screen. A period of 5 minutes was allowed for the fish to work themselves off this screen by their own efforts. Animals failing to do this were stimulated by tapping on the caudal peduncle with a glass rod. Electrical stimuli, tried initially, were generally found to be inadequate. Velocities were again raised by increments of about 1 cm/sec until no further amount of stimulation produced escape from the downstream screen. The end point of each determination was taken as that velocity at which the fish could maintain position for a period of 10 seconds at a distance of two body lengths from the downstream screen. The values obtained are considered to be equivalent to cruising speeds as recently defined by Brett, Hollands, and Alderdice (6).

The fork lengths of the tested animals were recorded, and the fish discarded.

(*2*) *Estimation of Total Body Levels of Chloride and Water*

Total body concentrations of chloride and water were determined on groups of 11–18 fish in fresh water and after 3½–4½, 10–10½, 14½, 23–24, 28, and 37–38 hours in sea water (salinity, 22–24‰).

Fish were killed by a sharp blow on the head, rinsed twice in distilled water, lightly blotted with paper towelling, weighed, dried to constant weight at 103° C, and powdered. Chloride determinations were carried out on the

[4]Leupold and Stevens Instruments Inc., Portland, Oregon.

powdered samples using the modified "open Carius" procedure proposed by J. F. Manery (1955, personal communication). In this technique samples are soaked overnight in silver nitrate, digested in nitric acid over a boiling water bath, and the residual silver nitrate estimated by thiocyanate titration. Digestion tubes were closed with glass bulbs having downward-drawn tips to prevent the possible loss of volatile chloride-containing substances formed during the process of digestion (22).

Fork lengths and body weights were recorded for each fish.

Results

(1) Cruising Speeds in Fresh Water

Cruising speeds of chum fry in fresh water, together with representative data on the performance of related species of salmonids in the same medium, are recorded in Fig. 2. A linear relationship between cruising speed (cm/sec) and fork length (mm) was apparent (b = 1.05, Table I). Data provided by Davidson (9), Bainbridge (1), and Brett, Hollands, and Alderdice (6) strongly suggest that such a relationship is characteristic of many, and perhaps most, species of fishes. Values for cruising speed obtained in this investigation agreed reasonably well with those already in the literature despite major differences in the techniques used in their estimation. Thus, Davidson (9) has recorded speeds of 13.1, 16.7, and 21.6 cm/sec for Atlantic salmon of fork lengths 3.2, 4.3, 5.2 cm respectively, based on observations of fish swimming in circular pools. Similarly, Brett, Hollands, and Alderdice (6) report mean cruising speeds of 25.5 cm/sec for underyearling coho salmon fry (mean fork length, 4.85 cm) and 25.3 and 28.3 cm/sec for underyearling sockeye salmon (mean fork lengths 6.23 and 7.64 cm respectively). The latter determinations were carried out in a Fry-type annular chamber under the same temperature conditions as those used in the present investigation.

(2) Cruising Speeds in Sea Water

Sample sizes, acclimation periods, mean fork lengths, and cruising speeds, as well as sea-water to fresh-water cruising-speed ratios for each experimental group, are summarized in Table I.

TABLE I

Experimental data. Effects of sea water on cruising speed

Hours in sea water (22–24‰)	Sample size	Mean fork length, mm	Mean cruising speed, cm/sec	Sea-water/fresh-water cruising-speed ratio
Fresh water	17	44.2	26.0	
3½– 4	10	45.7	23.6	0.849
9 –10	10	46.2	22.1	0.786
13 –14	10	46.1	19.1	0.682
20 –22	10	52.0	27.5	0.804
34 –36	4	51.0	32.3	0.973
48 –54	9	51.8	31.6	0.929
76 –82	9	54.7	35.7	0.965

Regression equation: cruising speed by fork length, $Y = 1.05X - 20.5$ cm/sec.

The number of fish in each sample, the number of acclimation periods tested, and the time required for each individual determination combined to produce a lapse of several weeks between the beginning and end of the investigation. As may be seen from the steady increases in mean fork length (Table I) of consecutive samples a certain amount of growth took place during the period of testing. For this reason a growth correction procedure was employed to provide a basis for comparison of cruising speed in fresh water and sea water. As will be indicated below, this correction is an approximation since size per se appeared to influence the adaptability of the animals to sea water. A regression equation was calculated for the variation of cruising speed with fork length for the group of 17 fish tested in fresh water (Fig. 2.) The mean lengths and cruising speeds of the individual experimental groups were then taken as the best established points, and sea-water cruising speed expressed as a ratio with the cruising speed which might be expected of similar-sized fish in fresh water (column 5, Table I). The latter values were interpolated from the regression equation based on values obtained in fresh water.

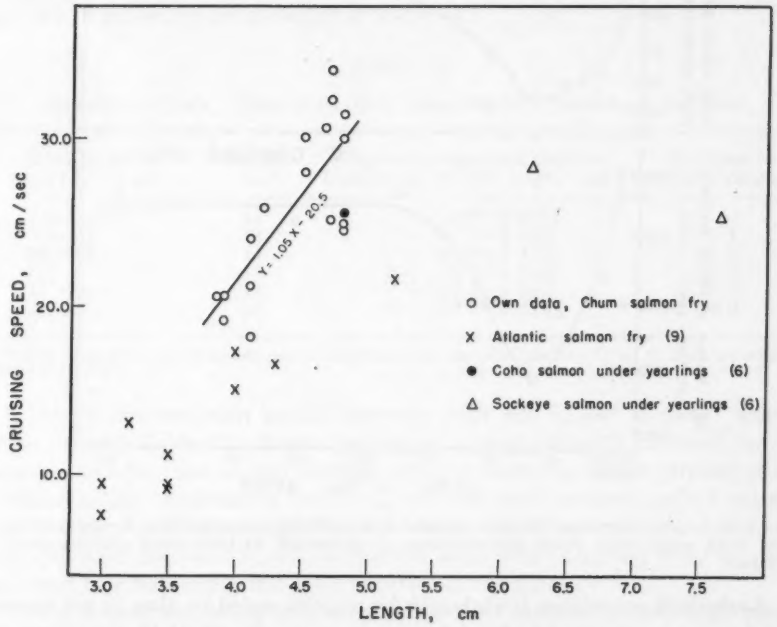

FIG. 2. Cruising speeds of chum salmon fry in fresh water. Representative data for Atlantic salmon fry (9), and for underyearling sockeye and coho salmon (6).

The data indicate that cruising speed was depressed immediately after transfer into sea water, and that this depression reached a maximum 14½ hours after transfer (Fig. 3). At that time the sea-water/fresh-water cruising-speed ratio was 0.682. Recovery from the effects of sea water was apparent

after 22 hours, and essentially complete by 36 hours. Between 36 and 80 hours after transfer there was no consistent increase in performance. The mean of the points at 36, 50, and 80 hours was 0.952, indicating that recovery was not complete within the duration of the experiment.

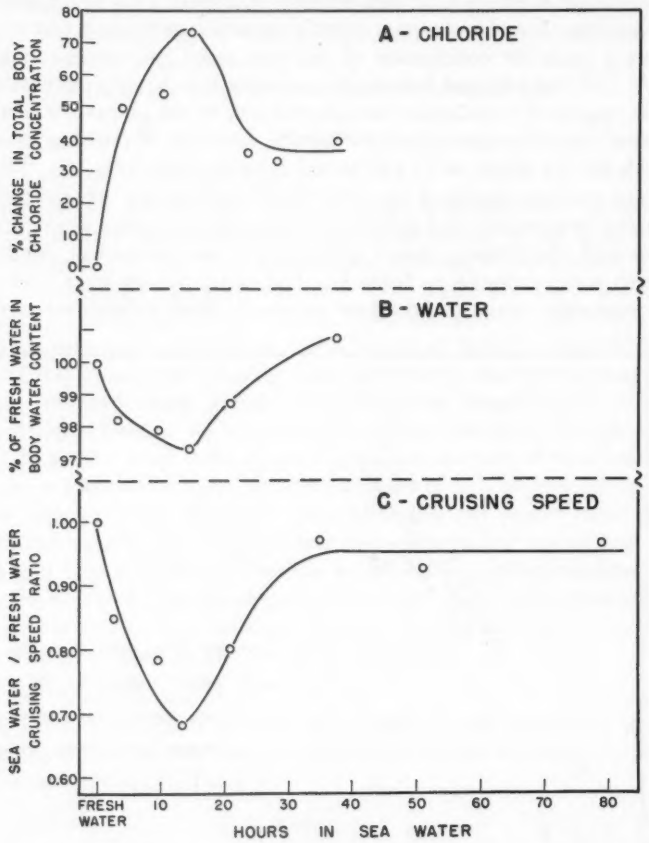

FIG. 3. A—percentage changes in total body chloride concentration; B—percentage of fresh water/body water concentration; C—sea-water to fresh-water cruising-speed ratios.

Analysis of covariance (fork length by cruising speed by time in sea water) carried out on the total body of data gave an F value of 24.37, significant at the 0.01 level of significance (25). The depression of swimming speed was, therefore, highly significant. Similar analysis of data from groups in fresh water, and after 36, 50, and 80 hours in sea water, gave an F value of 4.22. This value was significant at the 0.05 level. Since there was no consistent increase in performance over the latter period the data strongly suggest that sea water had not only an immediate and marked depressive influence, but

also that over the experimental period a continuing depression was present. Despite a considerable degree of recovery from the initial influence of sea water the fish never fully regained their fresh-water cruising speed.

While the sample sizes were not large enough to allow firm conclusions to be drawn there was some indication that size influenced the degree of depression of cruising speed. Taken as a percentage of the fresh water values the mean rates of decrease in cruising speed over the initial 14½ hours in sea water were −3.14, −2.48, and −2.08 per cent/hour for 40-, 50-, and 60-mm fry. Thus, it would appear that smaller fish were more markedly affected than larger fry. A similar conclusion may be drawn from the consideration of the cruising speed ratios derived for 40-, 50-, and 60-mm fish after 9 hours in sea water: 0.718, 0.815, and 0.852 respectively. This effect was consistent with the conclusion of Huntsman and Hoar (18) regarding the influence of size on the resistance to sea water displayed by Atlantic salmon fry and smolts.

(3) Total Body Chloride in Fresh Water and Sea Water

Sample sizes, regressions of chloride concentration on fork length, and periods in sea water are recorded in Table II.

TABLE II

Experimental data. Effect of sea water upon total body chloride concentration

Hours in sea water (22–24°/₀₀)	Sample size	Regression equations, chloride (mmole/kg) by fork length (mm)	% change in chloride concn.*
Fresh water	15	$Y = 110.3 - 0.602X$	
3½– 4½	15	$Y = 262.6 - 2.93X$	49.4
10 –10½	15	$Y = 240.9 - 2.37X$	54.1
14½	12	$Y = 278.5 - 2.83X$	73.2
23 –24	14	$Y = 220.4 - 2.22X$	32.9
37 –38	14	$Y = 229.3 - 2.33X$	39.0

*Values based upon chloride concentration interpolated from regression equations for fry of mean fork lengths recorded in Table I.

Chloride concentration varied inversely with size in fish in fresh water ($b = -0.602$, Table II). Since chloride ion is predominantly extracellular in position and does not readily undergo complex formation under physiological conditions (20), decreases in total body chloride level probably reflect reduction of the extracellular compartment in larger fish. Data on steelhead trout (17) indicated decreases in muscle chloride space with increased body weight, and tend to substantiate this interpretation of the data.

Transfer into sea water was accompanied by massive increases in total body chloride concentration, suggesting enlargement of the extracellular compartment, with concomitant cellular dehydration. Maximum concentrations were found 14 hours after transfer of the fry and coincided, therefore, with the period of maximum depression of cruising speed. Between 14 and 28 hours after transfer chloride levels fell smoothly. The final concentration at 36 hours was somewhat above that estimated for fry in fresh water. As was the case for steelhead trout (17) the data tend to corroborate Gordon's (14)

suggestion that salmonid fishes, in contrast to some other diadromous species, osmoregulate at two different levels, one characteristic of fresh water and the other characteristic of sea-water-adapted animals.

Investigations of Black (2) on chum salmon fry indicated a substantially similar pattern of events. Marked increases in chloride concentration occurred following transfer of fry into sea water of about the same salinity (27‰). Peak concentrations were found 12 to 15 hours after transfer, and relatively stable values were reached within 36 hours. Again the chloride concentrations of sea-water-adapted fry were above those of fish in fresh water. Marked differences were apparent, however, between the actual values reported by this author and those found in the present investigation. The source of this discrepancy may lie in the techniques employed in tissue chloride analysis. Shenk (22) has compared various procedures used in this analysis and has found that those utilizing *closed* digestion chambers (such as were used in the present study) consistently gave much higher chloride yields than those in which *open* digestion procedures were employed. Shenk has suggested that chloride loss in the latter case might be related to the formation and subsequent loss of volatile chloride-containing compounds, principally those of the chloranile type. Since closure of the digestion tubes would presumably preclude loss of volatile substances it would seem reasonable to attribute to this technical difference the variations in chloride values in the present and in Black's investigations.

Size, as indicated by fork length, had a marked influence over both the rate of increase and amount of chloride taken up by the fry. Higher rates of uptake (estimated as the mean net change in chloride concentration over the initial 14 hours in sea water) were characteristic of smaller fish. The mean net rate of change for 40-mm fish was 5.45 mmole/kg/hour. The corresponding value for 60-mm fry was 2.37 mmole/kg/hour. Again, the data bear out the conclusions of Huntsman and Hoar (18) regarding the relation of salinity tolerance to size in Atlantic salmon fry. Similarly, percentage changes in electrolyte concentration were much greater for the smaller than for the larger fish. The maximum level determined for 40-mm fry was 91.6% above the fresh-water concentration, while that for 60-mm fish represented an increase of only 46.4%.

In steelhead trout, ability to adjust rapidly to sea water appeared to be characteristic of a specific stage in growth (parr–smolt transformation) indicated by inflections in weight–length and weight–coefficient of condition relationships (17). No such changes were seen in the length–weight relationship of chum fry, or in the fry of a related species, the pink salmon (24). It would seem, therefore, that the ability of the chum salmon to rapidly adapt to sea water is a function of surface area to mass relationships, rather than one of particular physiological condition.

(*4*) *Total Body Water in Fresh Water and Sea Water*

Sample sizes, regression equations describing variation in water content with changes in fork length, and intervals in sea water are recorded in Table III.

TABLE III

Experimental data. Effect of sea water upon total body water concentrations

Hours in sea water (22–24‰)	Sample size	Regression equations, body water (g/kg) by fork length (mm)	% fresh water*/ body water level
Fresh water	18	$Y = 833.6 - 0.07X$	
3 – 3½	18	$Y = 822.2 - 0.06X$	98.2
9 –10	17	$Y = 820.7 - 0.06X$	97.9
14½	11	$Y = 813.2 - 0.29X$	97.3
22 –23	16	$Y = 825.6 - 0.01X$	98.7
37 –38	14	$Y = 842.0 - 0.08X$	100.8

*Values based upon body water concentrations interpolated from regression equations for mean fork lengths recorded in Table I.

A relationship between body weight and total body concentration was apparent in both fresh-water- and sea-water-acclimated animals. In general, changes in water content with weight resembled those for total chloride concentration. They were, however, much less marked than those indicated for the electrolyte.

Entry into sea water was accompanied by a decrease in total water level. The maximum effect was again found at 14 hours, and amounted to a decrease of about 3% from the original fresh water level. The values reported by Black (2) are comparable to the changes found in this investigation.

(5) *Correlation of Changes in Performance and Total Body Chloride and Water Levels*

Maximum chloride and minimum water concentrations coincided with maximum depression of locomotor performance. In the correlation of changes in these three variables, chloride and water have been related to the same size groups upon which Fig. 3 was based, and expressed as percentage changes from fresh-water levels.

The coefficient of correlation (25) between percentage changes in swimming speed and chloride concentration was 0.815 (significant at between the 0.03 and 0.01 levels). The coefficient of correlation between swimming speed and total body water concentration was 0.859 (significant at the 0.05 level).

Discussion

The data indicate that transfer of juvenile chum salmon fry from fresh water into sea water produces a depression of activity. This depression was divisible into two stages. The initial marked, but transitory, effect was significantly correlated with the adjustive phase of the osmoregulatory adaptation of the animals to sea water. The second phase was characterized by a continuing depression of much smaller magnitude present in animals which had apparently made a successful conversion from fresh water to marine osmoregulatory processes.

Several factors may be involved in this depressive influence of sea water: inhibition of electrolyte-sensitive components of the neuromuscular apparatus as a result of changes in anion and cation concentrations in cellular and

extracellular fluids; enhanced expenditure of metabolic energy in the re-establishment and maintenance of regulated water and electrolyte conditions; variations in intensity of the expression of specific behavioral patterns.

(*1*) *Inhibition of the Neuromuscular System During the Adjustive Phase*

Studies carried out on steelhead trout (17) indicated the following departures from regulated conditions during the adjustive phase of adaptation to sea water: (1) plasma and extracellular fluid levels of chloride, sodium, and potassium increased, (2) plasma ratios of chloride to sodium fell, (3) cellular volumes fell as a result of transfer of cellular fluids to the extracellular phase, and of loss of water to the environment, and (4) cellular concentrations of calcium, potassium, and chloride increased. These findings are summarized in the accompanying Yannet-Darrow diagram (Fig. 4).

If the data can be considered as characteristic of changes in other salmonids transferred from fresh water into sea water certain suggestions can be made as to the possible inhibition of the neuromuscular apparatus by changes in the concentrations of specific electrolytes.

Variations in potassium concentration, for instance, while they have been shown to influence muscular efficiency (11, 26), do not appear to have been great enough to have had any marked effect in this instance. Similarly, the ratio of the intracellular to extracellular potassium concentration, which is known to affect neuromuscular irritability (10), did not change greatly in the fish during the adjustive phase.

On the other hand, variations in bicarbonate level suggested by changes in plasma chloride to sodium ratios and by the work of Fontaine and Boucher-Firly (12) are believed to depress muscular activity (7), possibly through the effect of changes in cellular pH on enzyme activity.

With regard to calcium, Bowen (5) has found that small variations in the concentration of this cation profoundly inhibited the shortening of myosin fractions. The more recent and extensive work of Ranney (21) has shown that virtually all variations in free cation levels depress the response of myocardial fibers to adenosinetriphosphate. Since cellular calcium concentrations rose by over 50% in steelhead during their adaptation to sea water it would seem reasonable that a calcium-induced inhibition of muscle-fiber components may have occurred.

While magnesium was not studied in the investigation on steelhead trout the enrichment of this cation in sea water relative to fresh water would tend to promote its uptake by fish. Since increased magnesium concentrations are known to inhibit the release of the presynaptic transmitter substance acetylcholine (10) a magnesium-based contribution to impaired muscular efficiency may also be considered possible.

The data cited above suggest therefore that the inhibition of the neuromuscular apparatus by changes in electrolyte concentration in cells and extracellular fluids may operate as a factor of some importance in the depression of locomotor performance during the adjustive phase of adaptation to sea water.

(2) Effects of Variations in 'Scope for Activity' During and Following the Adjustive Phase

Fry (13) has suggested that the amount of energy available for behavioral activities such as swimming is a function of the animal's "scope for activity". This "scope for activity" is defined as the difference between the maximum rate at which energy sources can be converted into utilizable energy (active metabolic rate) and the rate at which the latter must be expended in vegetative functions (standard metabolic rate). In several experimental studies this author has demonstrated a direct relationship between performance and

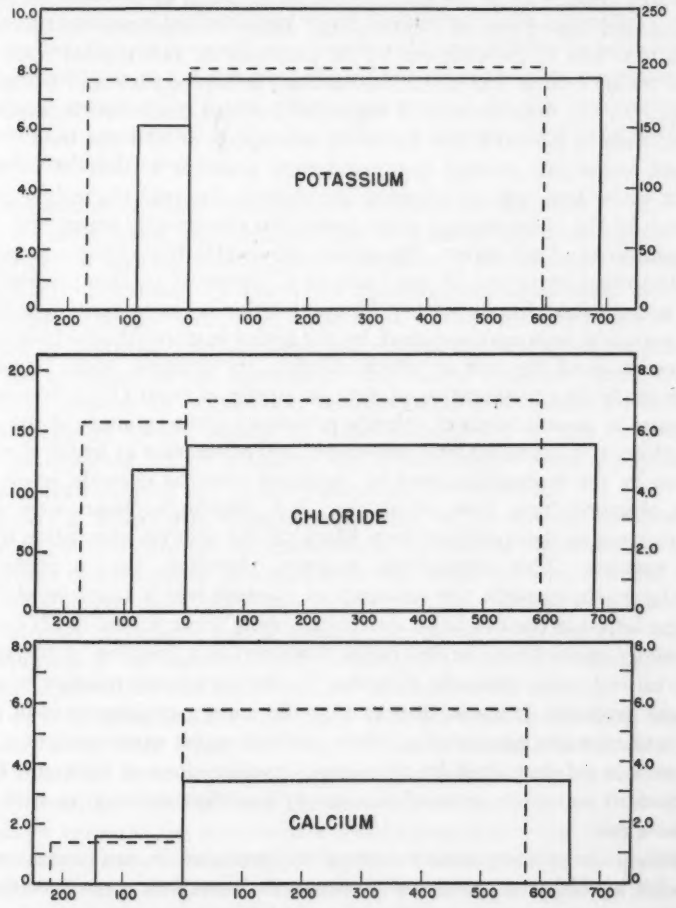

FIG. 4. Yannet-Darrow diagram describing variations in cellular and extracellular levels of chloride, potassium, and calcium of steelhead trout transferred from fresh water into sea water. Solid lines, values in fresh water; broken lines, values in sea water (chloride and potassium: 36 hours, calcium: 26 hours). *X* axis (left-side—chloride space; right side—tissue water minus chloride space), g/kg; *Y* axis, mmole/liter.

scope for activity. Depressed activity in fish adjusting to, or adjusted to, sea water may, therefore, be a reflection of a decrease in the difference between active and standard metabolic rates.

No data are at present available on changes in the "scope for activity" of salmonids during their adaptation to sea water. Consideration of the processes involved in osmoregulation in the two habitats may, however, provide some indication of their relative energy requirements.

The major features of osmoregulation in both fresh-water and marine habitats have recently been reviewed by Black (3). The central problem in fresh water appears to be the excretion of water taken up by osmosis, and the retention and absorption of electrolytes. Data accumulated by this author suggest that loss of chloride ion by all processes in two typical fresh-water species, *Salmo irideus* and *Carassius auratus*, is between 0.1 and 0.3 mmole/kg/hour. In the maintenance of regulated internal conditions an equivalent amount must be gained either by active absorption or with the food.

In sea water the central osmoregulatory problem is that of restricting osmotic water loss, and of reducing increases in internal electrolyte concentrations. In the alleviation of water losses fish customarily ingest and absorb large quantities of sea water. Since they are unable to produce a hypertonic urine the renal reduction of the "salt load" incurred in this process would result in a net loss of body water. This restriction is circumvented, at least as far as univalent ions are concerned, by the active extrarenal excretion of salts.

An estimate of the rate at which chloride, for example, must be excreted may be made by consideration of data on steelhead trout (17). The net rate of increase in muscle levels of chloride provides a gross estimate of the rate at which this ion is taken up from sea water, and of the rate at which it must be excreted in the re-establishment of regulated internal chloride levels. The values obtained from this calculation, 2–3 mmoles/kg/hour, were several times as great as those derived from Black (3) for chloride absorption by fresh water species. This comparison suggests, therefore, that a considerable disparity exists between the amounts of chloride which must be shifted in osmoregulation in the two habitats. Since Ussing's recent studies (27) indicate that energy expenditure in electrolyte transport is a function of the number of ions moved rather than the direction of their movement relative to electrochemical gradients it would appear that the energy requirements of marine osmoregulation are greater than those of fresh-water osmoregulation. This conclusion is substantiated by the recent investigations of Hickman (15) on the standard metabolic rates of flounder (*Platichthys stellatus*) in fresh water and sea water.

Increases in osmoregulatory energy expenditures in sea water may be paralleled by adaptive increases in maximum metabolic rate such that the scope for activity is not markedly changed following transfer into sea water. Several factors, however, mediate against this possibility. Fry (13) suggests that salmonids have a relatively restricted ability to increase their metabolic rates adaptively. In addition various investigators (see Fry (13) for review)

have demonstrated a direct dependency of metabolic rate upon the available oxygen concentration of the medium. Since the oxygen concentration of sea water is generally lower than that of fresh water it would seem unlikely that, without extensive resort to anaerobic metabolism, adaptive increases in maximum metabolic rate could counteract a decrease in "scope for activity" due to enhanced energy demands of marine osmoregulation. That anaerobiosis is not a particularly well-developed adaptive feature in salmonids has been indicated by Fry (13) and more recently by Blazka (4).

Enhanced utilization of metabolic energy in osmoregulatory processes may be considered, therefore, as a second possible mechanism to aid in accounting for the depression of performance of fish transferred into sea water.

Of the two factors it would seem reasonable to attribute to both inhibition and decreased "scope for activity" the initial and more pronounced depression of cruising speed. The latter effect may account for the continuing slight depression characteristic of the fry following the re-establishment of stable body electrolyte levels.

(*3*) *Variation in Intensity of Expression of Rheotactic Response*

On the other hand, the apparent loss of cruising speed during the 36- to 80-hour period may be attributed to a decrease in the intensity of expression of rheotactic responses. Under such circumstances behavioral as well as physiological changes should be considered. With the exception of values provided by Davidson (9) most determinations of cruising speed are dependent upon the tendency of the animals to maintain a constant visual field (e.g., 1, 6, 13). Should the motivation for this behavioral system be decreased, reduced cruising speeds, similar to those encountered in this investigation, might be expected. Such a change would presumably have a physiological basis although possibly not of the types already considered.

Prof. W. S. Hoar, who suggested this idea to the author, has been kind enough to provide data on the unstimulated activity of chum salmon both in fresh water and following acclimation to sea water for 4 to 6 weeks (W. S. Hoar, unpublished data, 1957–58). Schools of about 20 fish were observed in small annular chambers, and a measure of spontaneous activity made by determination of the number of fish crossing an arbitrarily chosen line during 5-minute observation periods. Table IV summarizes his results.

The data substantiate the conclusion that the activity of chum salmon is decreased even after relatively long periods of acclimation to sea water. Observations on some behavioral patterns (schooling, maintenance of directional swimming) indicated that the intensity of expression of these systems of behavior varied in the two media as well as general activity. The possibility of a generalized behavioral change must, therefore, be considered in attempting to account for the prolonged depression of cruising speed observed in the present investigation.

While considering rheotactic responses of fish following transfer into sea water some comment should be made regarding early work by Daugherty and Altman (8) on this subject. These authors concluded that changes in salinity

produced a reversal of sign in response to water currents. More recent investigations by Keenleyside and Hoar (19), however, indicated that reversals of this type were readily induced by slight changes in water temperature (i.e. 2–3° C). The latter authors concluded that the behavior observed by Daugherty and Altman was in all probability produced by relatively small temperature variations rather than by major differences in salinity. The fact that fish of good condition invariably displayed strong positive rheotactic responses after transfer into sea water would tend to corroborate the views of Keenleyside and Hoar.

TABLE IV

Spontaneous "activity" of chum salmon fry in fresh water and in sea water (W. S. Hoar, unpublished data, 1957–58)

Conditions	Temperature, °C	Observation periods	Mean "activity" values	
			Fresh water	Sea water
Fish placed in channels in daylight; observations begun immediately	11.0	8	201	47
	12.5	5	311	115
	11.0	4	137	23
	12.5	6	206	142
	12.5	17	278	129
Fish placed in channels in evening of day preceding observation	12.5	18	455	200
	12.5	20	409	205
	12.0	6	290	228
	13.0	10	408	328
	13.0	25	337	219
	13.0	23	422	102

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References

1. BAINBRIDGE, R. The speed of swimming of fish as related to size and to the frequency and amplitude of the tail beat. J. Exptl. Biol. **35**, 109–133 (1958).
2. BLACK, V. S. Changes in body chloride, density, and water content of chum (*Oncorhynchus keta*) and coho (*O. kisutch*) salmon fry when transferred from fresh water to sea water. J. Fisheries Research Board Can. **8**, 164–177 (1951).
3. BLACK, V. S. "Excretion & Osmoregulation". *In* The physiology of fishes. Vol. I. *Edited by* M. E. Brown. Academic Press, Inc., New York. 1957.
4. BLAZKA, The anaerobic metabolism of fish. Physiol. Zool. **31**, 117–128 (1958).
5. BOWEN, W. J. The effect of divalent ions on the shortening of myosin-B threads. Am. J. Physiol. **169**, 223–228 (1952).
6. BRETT, J. R., HOLLANDS, M., and ALDERDICE, D. F. The effect of temperature on the cruising speed of young sockeye and coho salmon. J. Fisheries Research Board Can. **15**, 587–605 (1958).

7. CREESE, R. J. Bicarbonate ion and striated muscle. J. Physiol. **110**, 450–457 (1949).
8. DAUGHERTY, A. M. and ALTMAN, L. C. Influence of hydrogen ion concentration, salinity and oxygen upon the rheotaxis of some marine fishes. Publs. Puget Sound Biol. Sta. **3**, 365–368 (1925).
9. DAVIDSON, M. V. Salmon and eel movement in constant circular current. J. Fisheries Research Board Can. **7**, 432–448 (1948).
10. ECCLES, J. C. The Physiology of Nerve Cells. John Hopkins Press, Baltimore. 1957.
11. FENN, W. O. Electrolytes in muscle. Physiol. Revs. **16**, 450–487 (1936).
12. FONTAINE, M. and BOUCHER-FIRLY, S. Recherches sur la reserve alcaline du sang des poissons. Ses variations au cours des changements de salinitie. Bull. Inst. Oceanog., Monaco, **646**, 1–12 (1934).
13. FRY, F. E. J. "The Aquatic Respiration of Fish". *In* The Physiology of Fishes. Vol. I. *Edited by* M. E. Brown. Academic Press, Inc., New York. 1957.
14. GORDON, M. S. Observations on osmoregulation in the Arctic char (*Salvelinus fontinalis*). Biol. Bull. **112**, 28–33 (1957).
15. HICKMAN, P. P. The osmoregulatory metabolism of the starry flounder, *Platichthys stellatus*. Doctoral thesis. University of British Columbia. 1958.
16. HOUSTON, A. H. Responses of juvenile chum, pink, and coho salmon to sharp sea-water gradients. Can. J. Zool. **35**, 371–383 (1957).
17. HOUSTON, A. H. Locomotor performance and osmoregulation in juvenile anadromous salmonids following abrupt environmental salinity change. Doctoral thesis. University of British Columbia. 1958.
18. HUNTSMAN, A. G. and HOAR, W. S. Resistance of Atlantic salmon to sea water. J. Fisheries Research Board Can. **4**, 409–411 (1939).
19. KEENLEYSIDE, M. H. A. and HOAR, W. S. Effects of temperature on the responses of young salmon to water currents. Behaviour, **7**, 77–87 (1954).
20. MANERY, J. F. Water and electrolyte metabolism. Physiol. Revs. **34**, 344–417 (1954).
21. RANNEY, R. E. Biochemical characteristics of glycerol-extracted myocardial fibers. Am. J. Physiol. **183**, 197–202 (1955).
22. SCHENK, W. D. Tissue chloride. Arch. Biochem. Biophys. **49**, 138–148 (1954).
23. SHEPARD, M. P. Responses of young chum salmon (*Oncorhynchus keta*, Walbaum) to changes in the sea water content of the environment. Master's thesis. University of British Columbia. 1948.
24. SKUD, B. E. Length–weight relationship in migrating fry of the pink salmon (*O. gorbuscha*) in Saskin Creek, Little Port Walter, Alaska. Copeia, No. 3, 204–207 (1955).
25. SNEDECOR, G. W. Statistical methods. 4th ed. Iowa State College Press, Ames, Iowa. 1946.
26. SZENT-GYORGYI, A. Chemistry of muscular contraction. 2nd ed. Academic Press, Inc., New York. 1951.
27. USSING, H. H. Ion transport mechanisms. Ciba Foundation Lect., University of British Columbia. 1958.

NOTES

BALANUS NUBILUS DARWIN

H. BARNES

Balanus nubilus was first described by Darwin (8). He had only limited material and no soft parts. There can be no question regarding his specific name, *nubilus*; it is so spelt in the text, classification, and description and titles of the plates. The species has been correctly named by some workers, notably Gruvel (9), Cornwall (2, 3, 4, 5, 6, 7), and Ricketts and Calvin (16); however, the Zoological Record (10, 11, 12) misquotes Cornwall giving the specific name as *nubilis*. Other workers, Pilsbry (15), Shelford *et al.* (17), Tait (18), and Henry (13, 14), have used *nubilis*. The error originates with Pilsbry (15), who had ample material at his disposal and who amplified considerably Darwin's description to include the large forms which occur from southern Alaska to Oregon. The species is common on the North American west coast and ecologists tend to turn to Pilsbry for their information since his is still the standard and outstanding monograph on American cirripedes. In pointing out that recently we have perpetuated this error (Barnes and Barnes (1)), Mr. I. E. Cornwall writes that in a letter to him Dr. Pilsbry wrote "...I do not know how it happened but, of course, it should be *B. nubilus*...". It appears desirable to draw attention to this error.

1. BARNES, H. and BARNES, M. The naupliar stages of *Balanus nubilis* Darwin. Can. J. Zool. **37**, 15–23 (1959).
2. CORNWALL, I. E. The barnacles of California (Cirripedia). Wasmann J. Biol. **9**, 311–346 (1951).
3. CORNWALL, I. E. The central nervous system of barnacles. J. Fisheries Research Board Canada, **10**, 76–84 (1953).
4. CORNWALL, I. E. Arthropoda 10e Cirripedia. Can. Pacific Fauna, **1**, 49 (1955*a*).
5. CORNWALL, I. E. The barnacles of British Columbia. Brit. Columbia Prov. Museum Education Handbook No. 7. 1955*b*.
6. CORNWALL, I. E. Identifying fossil and recent barnacles by the figures in the shell. J. Paleontology, **30**, 646–651 (1956).
7. CORNWALL, I. E. Identifying recent and fossil barnacles. Can. J. Zool. **36**, 79–89 (1958).
8. DARWIN, C. R. A monograph of the sub-class Cirripedia. Vol. 2. Balanidae. Ray Soc. London. 1854.
9. GRUVEL, A. Monographie des Cirrhipèdes. Paris. 1905.
10. HARDING, J. P. Crustacea. Zool. Record, **88**, Sect. 10 (1951).
11. HARDING, J. P. Crustacea. Zool. Record, **90**, Sect. 10 (1953).
12. HARDING, J. P. and INGLE, R. W. Crustacea. Zool. Record, **92**, Sect. 10 (1955).
13. HENRY, D. P. The Cirripedia of Puget Sound with a key to the species. Univ. Wash. Publs. Oceanog. **4**, 1–48 (1940).
14. HENRY, D. P. Studies on the sessile Cirripedia of the Pacific coast of North America. Univ. Wash. Publs. Oceanog. **4**, 95–134 (1942).

15. PILSBRY, H. A. The sessile barnacles (Cirripedia) contained in the collections of the U.S. National Museum; including a monograph of the American species. U.S. Natl. Museum Bull. No. 93, 1–366 (1916).
16. RICKETTS, E. F. and CALVIN, J. Between Pacific tides. 3rd ed. Stanford Univ. Press, Stanford. 1952.
17. SHELFORD, V. E., WEESE, A. O., RICE, L. A., RASMUSSEN, D. I., and MACLEAN, A. Some marine biotic communities of the Pacific coast of North America. Part I. General survey of the communities. Ecol. Monographs, **5**, 248–332 (1935).
18. TAIT, J. Experiments and observations on Crustacea. Part VI. The mechanism of massive movement of the operculum of *Balanus nubilis*. Proc. Roy. Soc. Edinburgh, Part I, **45**, 42–47 (1925).

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